UV-VIS SPECTROSCOPY

LAMBDA 365

UV Express Software Users Guide

PerkinElmer

Release History

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I. Introduction

I-1. Overview

This manual provides step-by-step instructions for the use of UV Express software with a Perkin Elmer Inc. Double Beam UV-Visible Spectrophotometer.
UV Express software must be installed onto a Microsoft® Windows 7 or Window 8 operating system to function properly.

I-2. Specifications of UV Express

Operating Environment
Support Double-beam UV-Vis Spectrophotometer

· Lambda 365 UV-Visible Spectrophotometer

Control Accessories

· 8 Position Multi Cell Holder
· Water Jacketed 8-Position Multi-Cell Holder
· Advanced Transmission Holder
· Film Holder
· Variable Angle Transmission Holder
· Micro Cell Holder
· Test Tube Holder
· Variable Pathlength Cuvette Holder
· Auto Sipper System with Software Control
· Single Cell Peltier Holder (without heated reference or with heated reference)
· 6-Position Peltier Controlled Cell Changer (without heated reference or with heated reference)
· Fixed Angle Reflectance Holder
· Autosampler
· 50 mm Transmission / Reflectance Sphere
. Magnetic Stirrer Assembly (Auto Type)
. Magnetic Stirrer Assembly (Manual Type)

Computer Requirements

· Processor: Intel® Core 1.5 GHz or faster
· RAM: At least 1GB
· Hard disk: 50GB with 1GB free
· Input device : Mouse and keyboard
· Monitor: 1024x768 (minimum)
· Media: CD ROM Drive
· Port: USB port for the data acquisition

Operating System

· Microsoft® Windows 7 or Windows 8

Output Device

· Microsoft® Windows compatible printer

Scan Mode	
Scan Setup	· Measures Absorbance, Transmittance, Reflectance at the full or selected wavelength · Export to Clip board, CSV file, Excel file, BMP file, ASCII file, JCAMP file · Zoom In / Out, Add / Edit Labels
Equation Calculation	· Allows the user to enter support equations for the evaluation of the data · Supports Functions: +, - , /, *, ABS, Exp, LN, LOG10, SQRT
Find/Peak Valley	· Find peaks or valleys automatically or manually

Quantification Mode	
Quantification Standard/Sample	· Concentration Unit: All units user-specifiable · Fit Order: Linear, Quadratic, Cubic · Supports intercept of calibration curve · Calculation of correlation coefficient · Number of repeat measurements (1 to 5) to obtain a mean value

Kinetics Mode	
Time Based Kinetics	· Monitors AU, %T continuously over time at a fixed wavelength · Time Unit: Min, Sec · Zero Order, Initial Rate, First Order, Delta AU
Wavelength Program Mode	
Wavelength Program	· Measure Absorbance, Transmittance, Reflectance at selected multiple wavelengths
Bio Mode	
Nucleic Acid Analysis	· General ratio with two wavelengths for the calculation of user specified ratios · Determine concentration of protein and nucleic acid using coefficients · Baseline Correction
Protein Analysis	· Predefined methods · Bradford Protein Analysis at 595 nm · Bicinchoninate (BCA) at 562 nm · Biuret Protein Analysis at 540 nm · Lowry Protein Analysis high sensitivity at 750 nm · Lowry Protein Analysis low sensitivity at 500 nm · Lowry Protein Analysis at 740 nm · Trinitrobenzene Sulfonate at 416 nm
Cell Density	· Predefined methods · Cell Density calculated with absorbance of 600 nm
Thermal Denaturation	· Temperature Unit: ºC · Temperature limit: from -5 to 100 ºC · Tm calculated with average method & 1st Derivative · User defied equation allows calculation from Tm value (ex: %G-C)

Scanning Kinetics Mode	
Scanning Kinetics	· Monitors AU, %T and %R continuously over time at a specified wavelength range · Time Unit: Min, Sec · Zero Order, Initial Rate, First Order, Delta AU

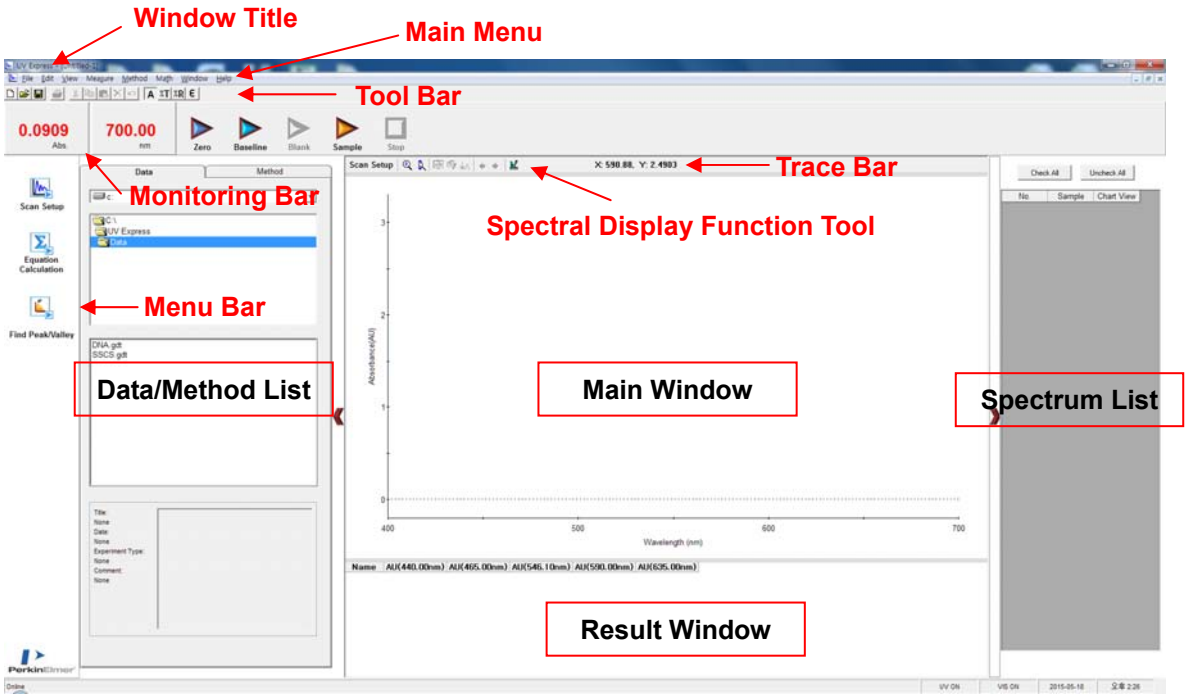
Scanning Quantification Mode	
Scanning Quantification	· Measures Absorbance, Transmittance, Reflectance at the full or selected wavelength · Concentration Unit: All units user-specifiable · Fit Order: Linear, Quadratic, Cubic · Supports intercept of calibration curve · Calculation of correlation coefficient

Multi-Component Analysis Mode (Optional)	
Multi-Component Analysis	· Analyze complex compounds containing multiple components (up to 4 components) · Define the concentration of each component.

System Performance Validation Software (Optional)	
Validation	· Automatic Validation Wizard assists with the validation across the UV and Visible range · Includes Photometric, Wavelength, Resolution, Stray Light, Noise and Stability

I-3. UV Express Software Interface

This chapter describes the unique display features of UV Express Software.

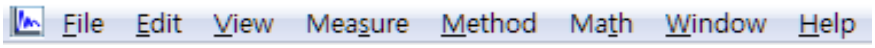


I-3-1. Window Title

Display the title of an active window or file. If data is saved as a specific file, its name will become the window title; otherwise, the title will be assigned automatically as [Untitled-1], [Untitled- 2], etc.

I-3-2. Main Menu

The Main Menu consists of a File Menu, Edit Menu, View Menu, Measure Menu, Method Menu, Math Menu, Window Menu and Help Menu.



I-3-3. Menu Bar

Create the icons of Scan Setup, Equation Calculation or Find Peak/Valley.

I-3-4. Toolbar

The toolbar provides quick access to basic commands without opening a menu. Users can modify the configuration of the toolbars as desired.

Icon	Command	Hot Key	Icon	Command	Hot Key
	New	Ctrl + N		Delete	Del
	Open	Ctrl + O		Undo	Ctrl + Z
	Save	Ctrl + U		Absorbance	
	Print	Ctrl + P		Transmittance	
	Cut	Ctrl + X		Reflectance	
	Copy	Ctrl + C		Energy	
	Paste	Ctrl + V			

I-3-5. Main Window & Spectral Display Function Tool

Display the spectral data. If there are several spectra in the window, only an active red spectrum can be edited.

Display the spectrum window and calibration curve in the quantification experiment.

Display a main window as required.

The following table provides a brief description of each Display Function Tool.

Toolbar	Command	Description
	Zoom Reset	Reset the zoom area to the original size
	Y Axis Auto Scale	Allow the auto scale of Y-axis depending on the measurement result
	Pick Peak	Look for peaks. Use in Find Peak/Valley Mode
	Pick Valley	Look for valleys. Use in Find Peak/Valley Mode
	Cursor	Display the cross lines for selecting data points
	To Left	Move the cursor to the left
	To Right	Move the cursor to the right
	Add Label	Add label on the spectrum.

	Edit Label	Edit label on the spectrum.
	Properties	Display Interval, Change a chart (background & axis) color, legend display, grid, X-axis and Y-axis scale, and decimal point format, etc.

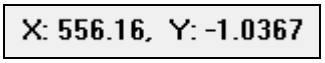
See **XI. Display Function Tools** for more details.

I-3-6. Result window

Display result values of performed measurements.

I-3-7. Trace bar

Display X-axis and Y-axis values of the mouse pointer on the spectrum.



I-3-8. Monitoring Bar

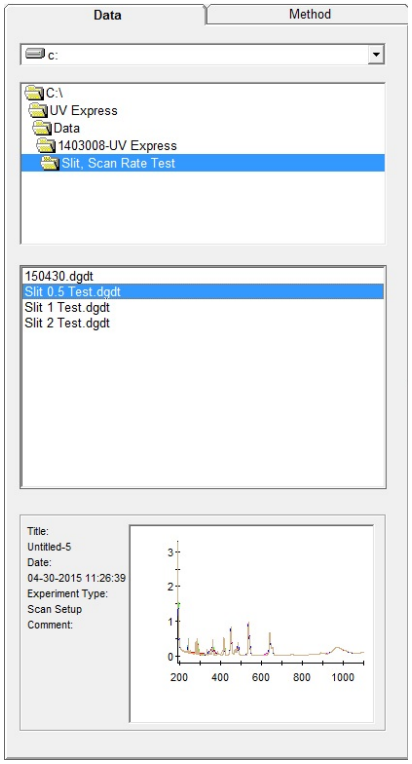
Display the current wavelength and photometric value.

Common functions for measurement.

Icon	Command
	Displays the current photometric value
	Displays the current wavelength
	Set the absorbance value(or transmittance) of the current wavelength to Zero (100%T for transmittance)
	Measures data for baseline correction
	Measures data for blank correction
	Collects a sample spectrum
	Stops measuring data

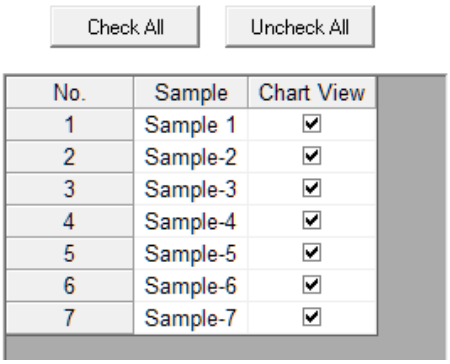
I-3-9. Data/Method List

Search and select the saved Data and Method in folder trees.



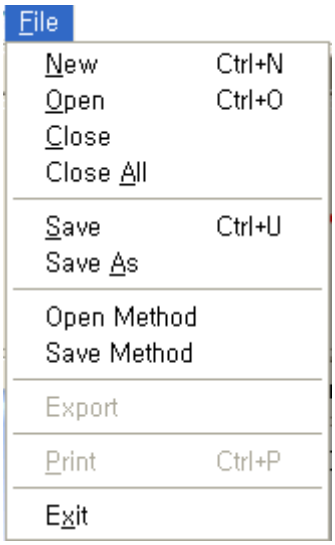
I-3-10. Spectrum List

Check the spectrum that displayed on the Main Window.



II. File Menu

The File menu includes commands to perform general file functions as shown in the following table.

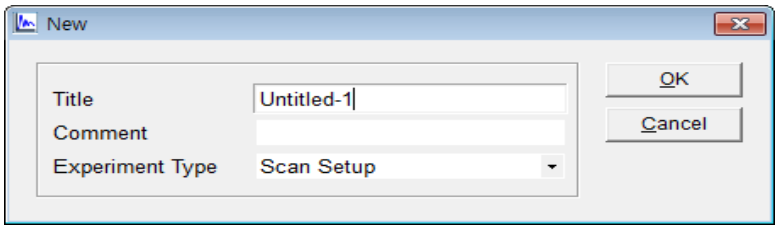


Command	Function
New	Open a new window
Open	Open saved data
Close	Close the current window
Close All	Close all windows
Save	Save data
Save As	Save data using a new file name
Open Method	Open a saved method
Save Method	Save a method
Export	Export data to another program
Print	Print results
Exit	Exit UV Express Software

II-1. New

- Use the New command to open a new window.
- Procedure

1. Click **New** to open a new window.



2. Enter a **Title** and **Comment** (if desired) and select the **Experiment Type**. If you do not enter a title, the title will be assigned automatically as [Untitled-1], [Untitled-2].....

3. Click **OK.**

II-2. Open

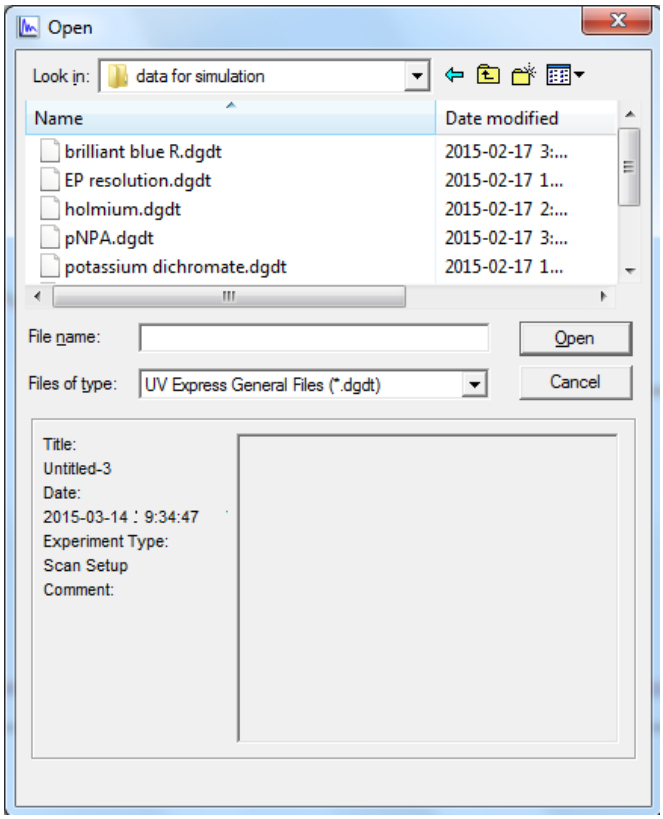
- Use the Open command to open data in a file.
- Procedure

1. Select a file to open, the title, date, comment, experiment type and spectrum is displayed.

2. Select **Open**.

3. To open several sets of data in the same window, hold down either the **Shift** or **Control** key, select the files, and click **OK**.

4. The selected files can be displayed in a new window by clicking **Open in New Window** at the bottom of the box.



The following file type options are available:

File Type	Description
UV Express General Files (*.dgdt)	Scan Setup data
UV Express Quantification Files (*.dqdt)	Quantification data
UV Express Kinetics Files (*.dkdt)	Kinetics data
UV Express Wavelength Program Files (*.dsrdt)	Wavelength Program data
UV Express Scanning Kinetics Files (*.dskdt)	Scanning Kinetics data
UV Express Scanning Quantification Files (*.dsqdt)	Scanning Quantification data
UV Express Nucleic acid Files (*.dbdt)	Nucleic Acid data
UV Express Protein Analysis Files (*.dpdt)	Protein Analysis data
UV Express Cell Density Files (*.dcdt)	Cell Density data
UV Express Thermal Denaturation Files (*.dthdt)	Thermal Denaturation data
UV Express Validation Files (*.dvdt)	Validation data
UV Express Multi-Component Analysis Files (*.dmcdt)	Multi-Component Analysis data
Spectra (*.sp)	UV WinLab data

II-3. Close

- Use the Close command to close a window.
- Verify the data was saved before closing the window.

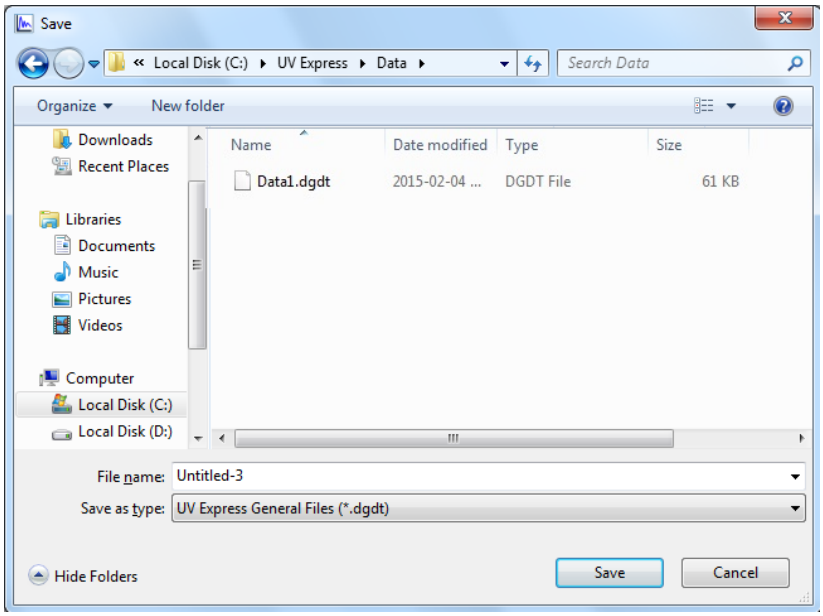
II-4. Close All

- Use the Close All command to close all windows.

II-5. Save

- Use the Save command to save the data in the current window.
- Procedure

1. To save data, select a folder to save data in, enter a file name, and click **Save**.

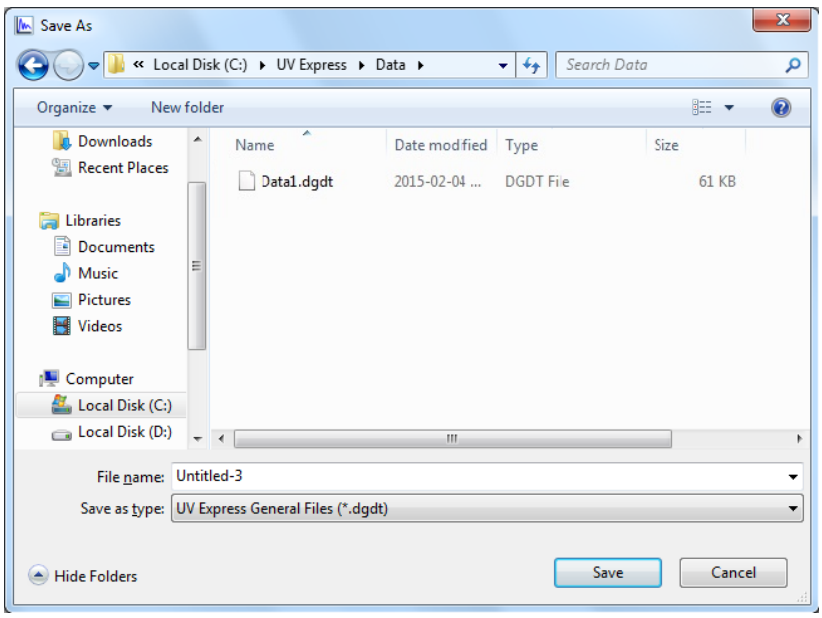


Please refer to **II-2. Open** for file types.

II-6. Save As

- Use the Save As command to save data using a new file name.
- Procedure

1. To save data with a new file name, select a folder to save the file in, enter a file name and click **Save**.

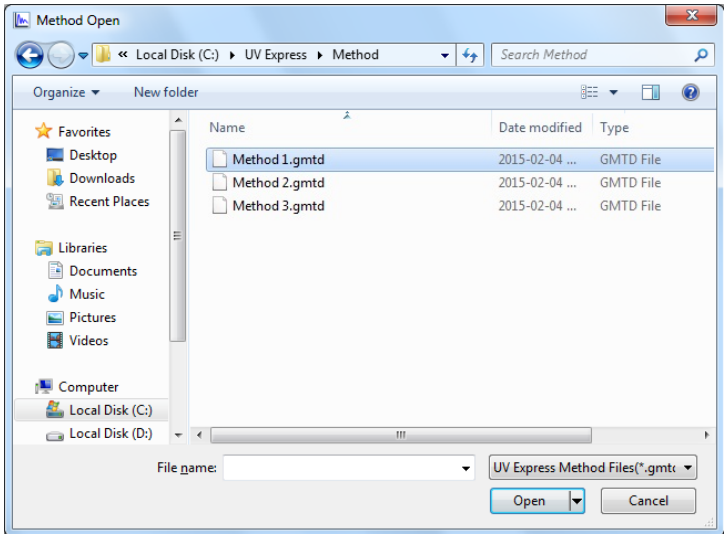


Refer to **II-2. Open** for file types.

II-7. Open Method

- Use the Open Method command to open a method stored in a file.
- Procedure

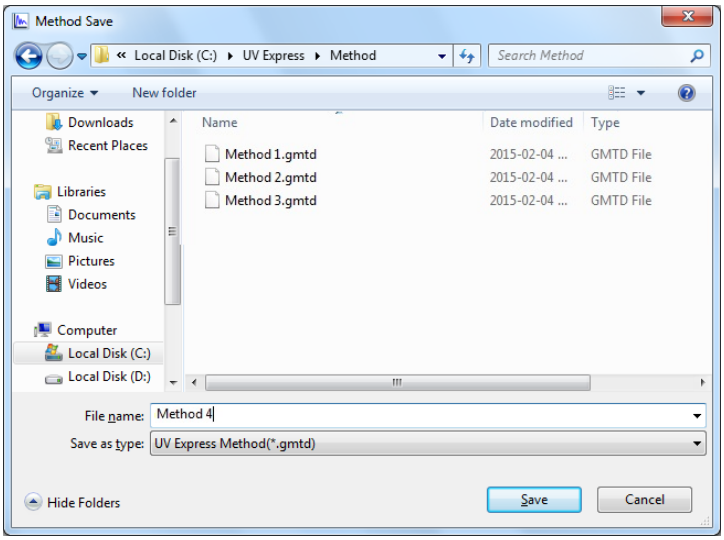
1. To open a method, select a file to open and click **Open**. The method file extension is ".gmtd".



II-8. Save method

- Use the Save Method command to save the current setting for the data collection and processing methods.
- Procedure

1. To save a method, enter a file name and click **Save**.

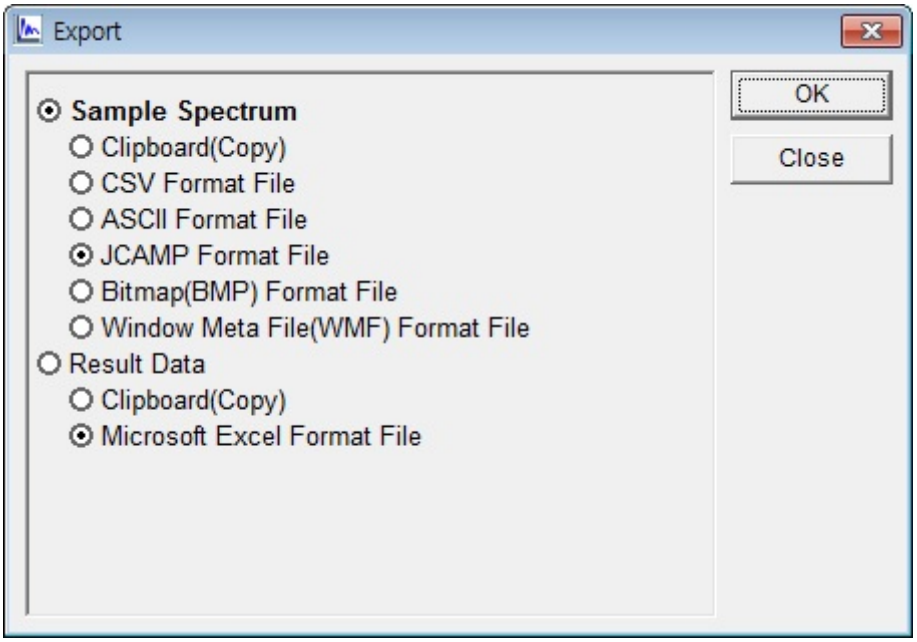


2. Method file extensions are automatically assigned as *.gmtd.

II-9. Export

- Use the Export command to export the data to another program such as Microsoft Excel, or other Windows programs.
- Procedure

1. Click **Export**. Choose the desired format type as shown below, and click **OK**.

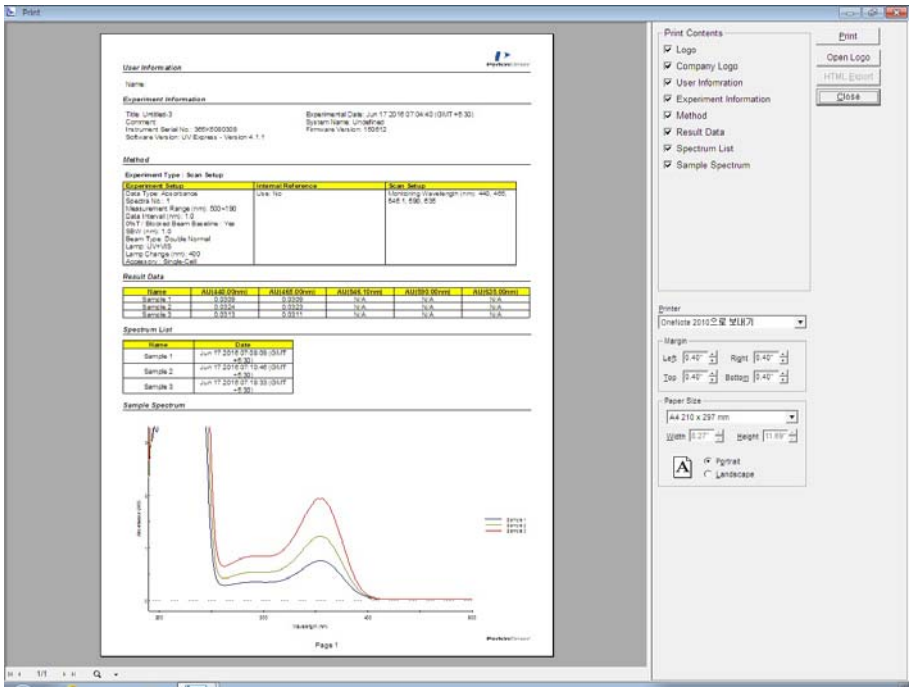


File Type	Description
Export Sample Spectrum	
Clipboard(Copy)	Copy the spectrum as the picture.
CSV Format File	Export a spectrum as a *.csv file. The interval between wavelengths is determined by the sampling interval.
ASCII Format File	Export a spectrum as a *.asc file.
JCAMP Format File	Export a spectrum as a *.dx file.
Bitmap(BMP) Format File	Export a spectrum as *. bmp file.
Window Meta File(WMF) Format File	Export a spectrum as *.wmf file.
Export Result Data	
Clipboard(Copy)	Copy the result data
Microsoft Excel Format File	Export the result data as MS Excel format file

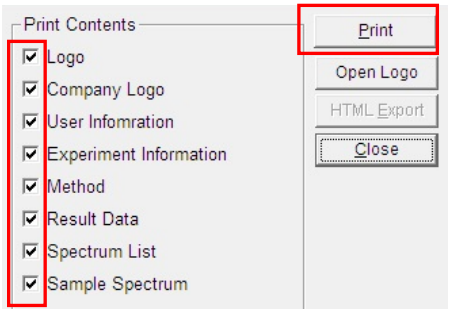
II-10. Print

- Use the Print command to print or preview data in the current window.
- Procedure

1. The following print-preview window will be displayed.



2. Select the required print contents and select **Print**.



Function	Description
Pre Page	Pre Page allows you to go back to the previous page
Next Page	Next Page allows you to go to the next page
Zoon In	Zoom In allow you to maximize the window
Zoom Out	Zoom Out allows you to revert the maximized the window to the standard size

NOTE: *User can change the company logo.*

a. *Click **Open Logo.***

b. *Select the desired company logo. The selectable logo file is *.bmp file and the recommended size is 110x50 pixels.*

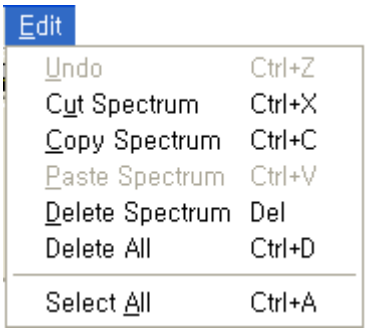
c. *Confirm the company logo is changed.*

II-11. Exit

- Use the Exit command to close UV Express Software.

III. Edit Menu

The Edit menu includes commands to perform spectrum functions as shown in the following table.



Command	Function
Undo	Undo previous
Cut Spectrum	Cut spectrum
Copy Spectrum	Copies spectrum
Paste Spectrum	Pastes the copied/cut spectrum
Delete Spectrum	Deletes selected spectrum
Delete All	Delete all selected spectra in a Window
Select All	Select all spectra in a Window

III-1. Undo

- Use the Undo command to undo a previous edit operation.
- Procedure

1. To undo an edit, click **Undo** in the Edit menu.

III-2. Cut

- Use the Cut command to remove the selected spectra and place the cut items on the clipboard.
- Procedure

1. Select a spectrum to cut.

2. Select **Cut** in the Edit menu. If there are several spectra in the window, select **Spectrum List** to choose a particular spectrum to cut.

3. Place the cut spectrum in another location using the **Paste** command.

III-3. Copy

- Use the Copy command to duplicate the selected items onto the clipboard.
- Procedure

1. Select a spectrum to copy and click **Copy** in the Edit menu.

2. Paste the copy into another location using the **Paste** command.

III-4. Paste

- Use the Paste command to place a cut or copied item in the desired location.
- Procedure

1. Cut or copy a spectrum to paste into a window.

2. Select **Paste** in the selected window. Copies of the same item can continue to be pasted until another item is cut or copied.

III-5. Delete

- Use the Delete command to delete the selected spectrum from the active spectrum window.
- Procedure

1. Select a spectrum to delete or click **Select All** to delete all spectra in the window.

2. Click **Delete**.

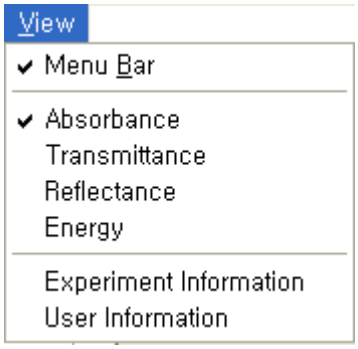
III-6. Select All

- Use the Select All command to select all spectra in the active spectral window.
- Procedure

1. To select all, select **Select All.**

IV. View Menu

- The View menu includes commands to change and customize software windows as show below.



Command	Function
Menu Bar	Create the icons of Scan Setup, Equation Calculation, Find Peak/Valley
Absorbance	Display the unit of Y-axis by absorbance
Transmittance	Display the unit of Y-axis by transmittance
Reflectance	Display the unit of Y-axis by reflectance
Energy	Display the unit of Y-axis by energy
Experiment Information	Display Experiment Information
User Information	Display User Information

IV-1. Absorbance

- Use the Absorbance command to convert the unit of the Y-axis to absorbance mode.
- Procedure

1. Click **Absorbance**, and the unit of the Y-axis changes to absorbance mode.

IV-2. Transmittance

- Use the Transmittance command to convert the unit of the Y-axis to transmittance mode.
- Procedure

1. Select **Transmittance** and the unit of the Y-axis changes to transmittance mode.

IV-3. Reflectance

- Use the Reflectance command to convert the unit of the Y-axis to reflectance mode.
- Procedure

1. Select **Reflectance** and the unit of the Y-axis changes to reflectance mode.

IV-4. Energy

- Use the Intensity command to convert the unit of the Y-axis to intensity mode.
- Procedure

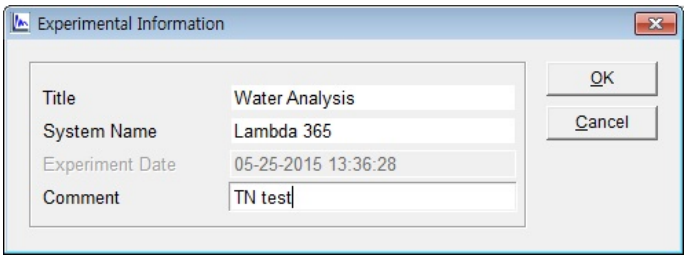
1. Select **Energy** and the unit of the Y-axis changes to energy mode.

IV-5. Experimental Information

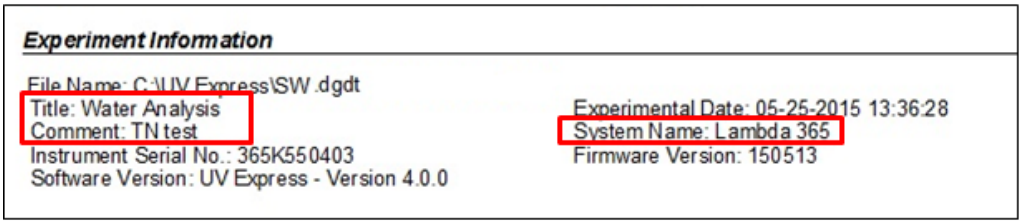
- Use the Experiment Information command to exchange the experiment information.
- Procedure

1. Click **Experiment Information**.

2. Enter **Title, System Name** and **Comment** information. A comment can include up to 80 characters. Select **OK**.



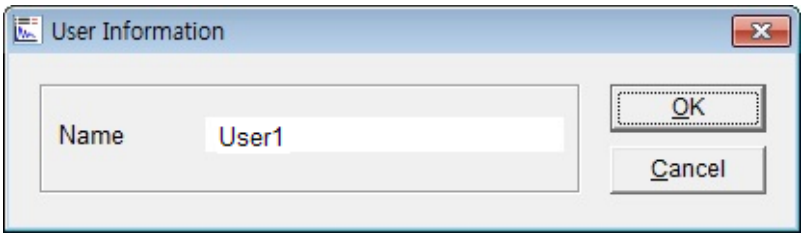
3. User can check the information at the Experiment Information contents of print window.



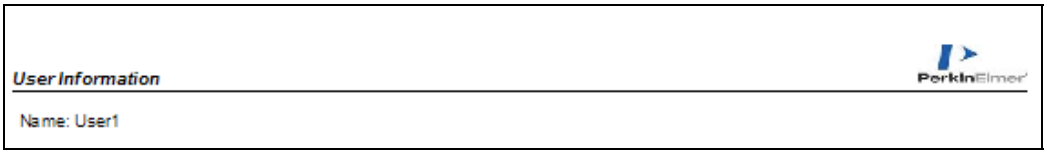
IV-6. User Information

- Use the User Information command to exchange the user information.
- Procedure

1. Click **User Information**. Enter Name information and select **OK**.

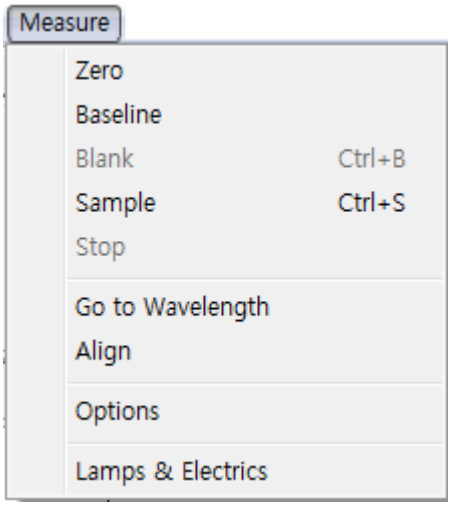


2. User can check the information at the User Information contents of print window.



V. Measure Menu

The Measure menu includes commands to perform measurement as shown in the following table.



Command	Function
Zero	Sets the absorbance value (or transmittance) of the current wavelength to zero (100%T for transmittance)
Baseline	Measure data for baseline correction
Blank	Collect a blank spectrum in case of single beam type
Sample	Collect a sample spectrum
Stop	Stop measuring
Go to Wavelength	Move the grating to the desired wavelength
Align	Move the grating when the accessory is used
Options	Select one of save methods and Baseline Display method
Lamps & Electrics	Check the lamp status

V-1. Zero

- Use the Zero command to set the absorbance (or transmittance) of the current wavelength to zero (100%T for transmittance).
- Procedure

1. Select **Zero**.

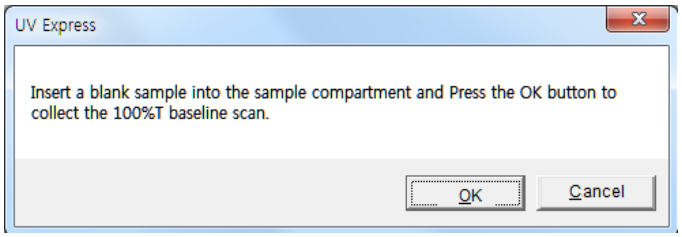
2. Change the absorbance of the current wavelength to zero.

V-2. Baseline

- Measure data to collect the 100%T baseline scan.
- Procedure

1. Select **Baseline**.

2. Insert blank solutions into the reference and sample holders or empty the cell holders. Select **OK**.



V-3. Blank

- Use the Blank command to collect a new blank spectrum in the single beam type.
- Procedure

1. Place a blank in the sample holder.

2. Select **Blank**.

Measure a new blank spectrum each time parameters for an experiment are changed.

V-4. Sample

- Use the Sample command to measure a sample.
- Procedure

1. Place a sample in the sample holder and a reference in the reference holder.

2. Select **Sample**.

This icon is activated after a Baseline is measured.

V-5. Stop

- Use the Stop command to stop data collection.
- Procedure

1. Select **Stop**.

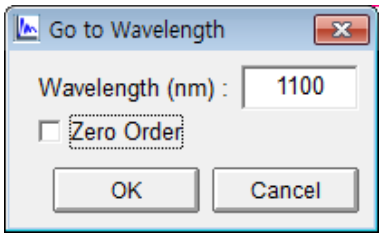
2. Stop measuring.

V-6. Go to Wavelength

- Move to the grating when the desired wavelength.
- Procedure

1. Select **Go to Wavelength**.

2. Set the desired wavelength and click **OK**.



- This is intended for service. Please uncheck the Zero Order.

V-7. Align

- Move to the grating when the accessory is used.
- Procedure

1. Click **Align**.

2. Move to the grating to the 0 nm.

V-8. Options

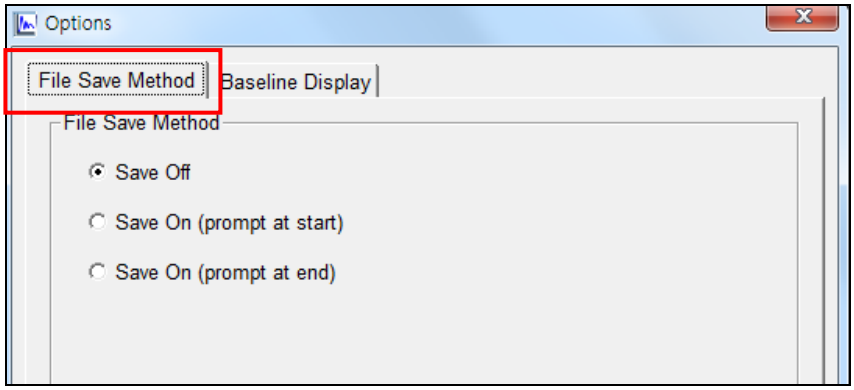
- Select one of save methods and Baseline Display methods.

V-8-1. File Save Method

- Save the method automatically or manually.
- Procedure

1. Select **Options** and **File Save Method** tab.

2. Select one of save methods. Select **OK**.



a. Save Off : User can save the desired data.

b. Save On (prompt at start): Display the windows Save As dialog box at the start of the collect where user can enter the file name for data.

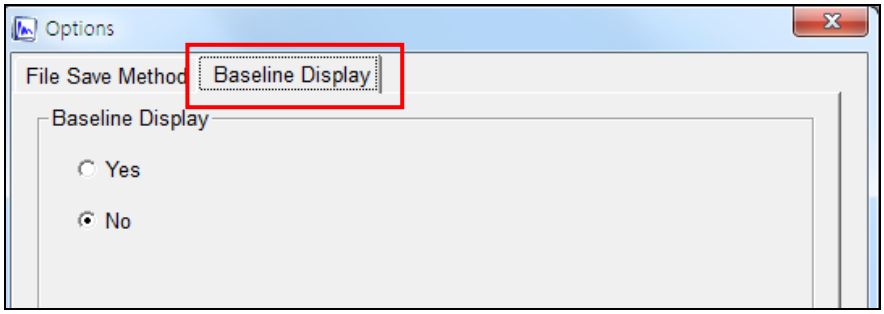
c. Save On (prompt at end): Display the windows Save As dialog box at the end of the collect where user can enter the file name for data.

V-8-2. Baseline Display

- Display Baseline scan data after measure sample.
- Procedure

1. Select **Options** and **Baseline Display** tab.

2. Select Yes or No. Click **OK**.

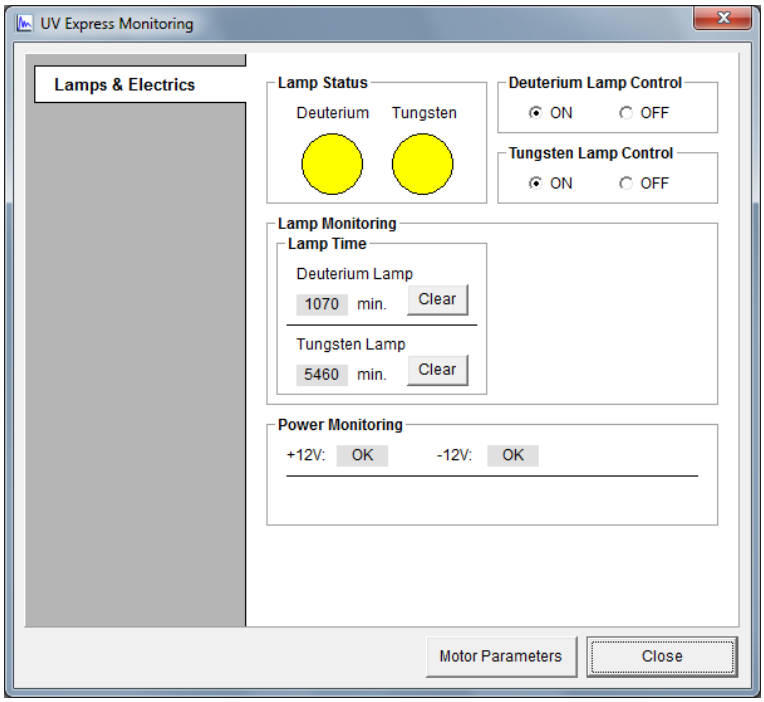


V-9. Lamps & Electrics

- Check the lamp status.
- Procedure

1. Select **Lamps & Electrics**.

2. Check the lamp status.



a. **Lamp Status**: Use to verify the ON/OFF status of the lamps. When lamp is turned on, the circle appears yellow. When it is turned off the circle appears white.

b. **Lamp Control**: Use to control ON/OFF status of the lamps. The user must wait until the process of updating the status is completed and disappears.

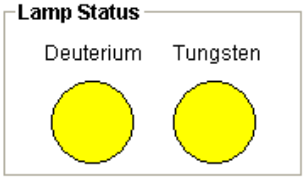
c. **Lamp Monitoring**:
Lamp Time: View the exhausted time of lamp. Select **Clear** to reset the time count only when installing a new lamp.

d. **Power Monitoring:** Use to check the power status.

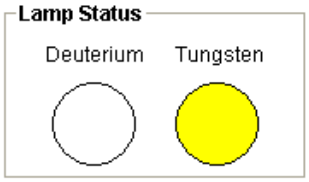
V-9-1. Lamp Status

Use to check the ON/OFF status of lamps. When lamp is turned on, the circle appears yellow. When it is turned off the circle appears white.

Example 1: Both lamps are turned on.



Example 2: Only the VIS (tungsten) lamp is turned on.

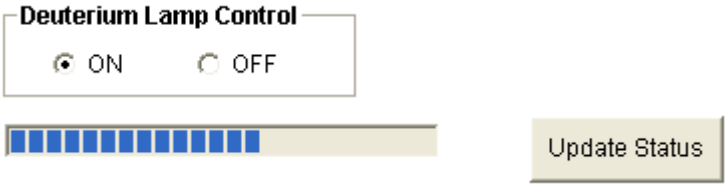


V-9-2. Lamp Control

Use to control the ON/OFF status of the lamps.

1. Select **On** in the Deuterium Lamp Control to turn on the Deuterium (UV)lamp. The user must wait until the process of updating the status is completed and disappears.

2. Select **OFF** in the Deuterium Lamp Control to turn it off.



3. Select **ON** in the Tungsten Lamp Control to turn on the Tungsten (VIS) lamp.

4. Select **OFF** in **Tungsten Lamp Control** to turn it off.

V-9-3. Lamp Monitoring

Use to check the lamp power and time used.

1. **Lamp Time**: View the exhausted time of lamp. Click Clear to reset the time count only when installing a new lamp.

Lamp Life Time:

- . Deuterium lamp: 2,000 hours
- . Tungsten lamp: 1,500 hours:

V-9-4. Power Monitoring

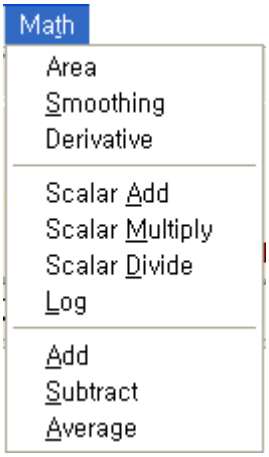
View status for the power of the instrument.

VI. Method Menu

- Setup parameters for the measurement.
- Refer to each mode in details.

VII. Math Menu

The Math menu includes commands to analyze the collected data.

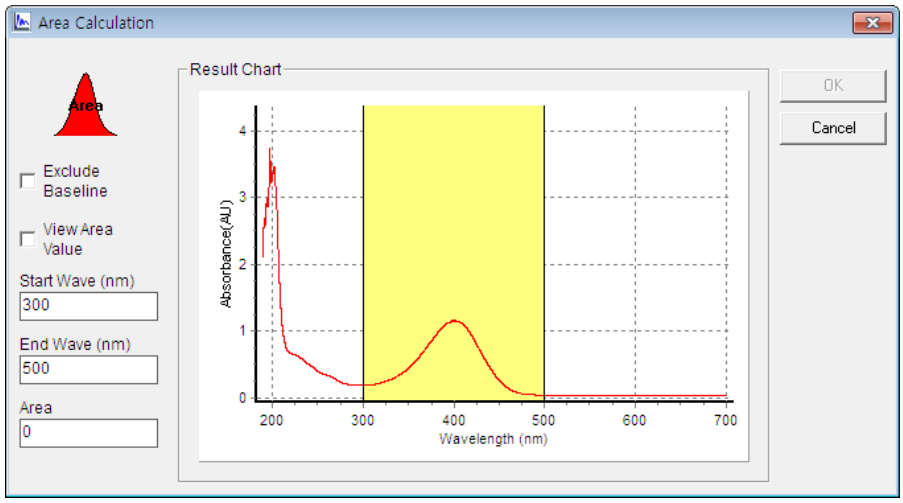


Command	Function
Area	Perform to calculate the area
Smoothing	Smooth the spectrum
Derivative	Obtain data after applying a derivative
Scalar Add	Add a constant value to y-value
Scalar Multiply	Multiply the y-value by a constant value
Scalar Divide	Divide the y-value by a constant value
Log	Compute the common logarithm of the y-value
Add	Obtain added data of two spectra
Subtract	Obtain subtracted data of two spectra
Average	Obtain the average data of spectra

VII-1. Area

- Use the Area command to calculate the are in the specified range
- Procedure

1. Select **Area**. The following dialog box is displayed.



2. Set the parameters.

   a. **Exclude Baseline:** Exclude the baseline from the calculated area value automatically.

   b. **View Area Value**: Display the area value in the result window.

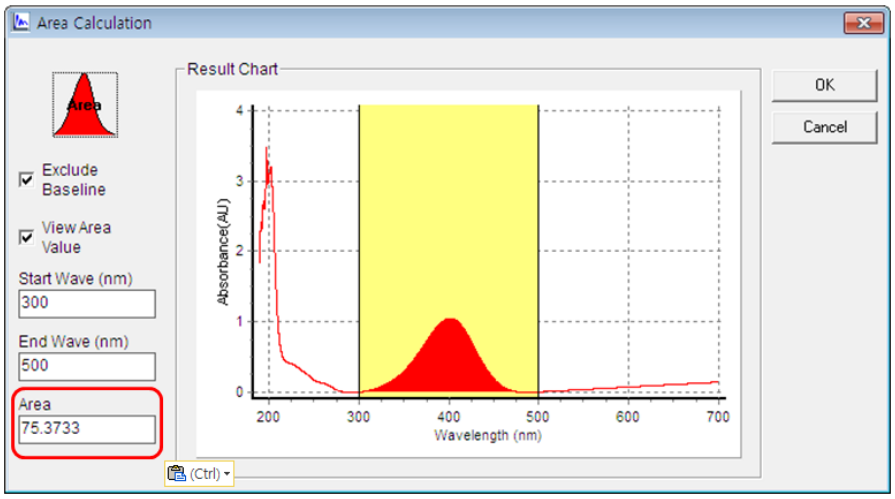
   c. **Start Wave (nm)**: Enter the start wavelength for the area calculation.

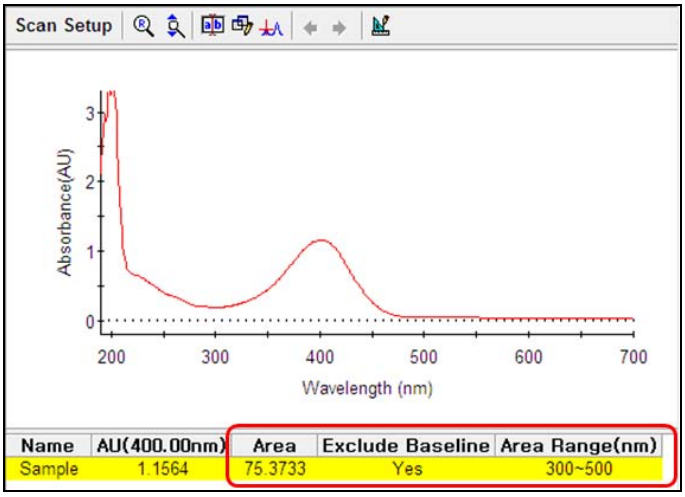
   d. **End Wave (nm)**: Enter the end wavelength for the area calculation.

   e. **Area**: Display the calculated value.

3. After entering the parameters, click the Area icon.

4. The calculated area and range are displayed in the Area tab. If you check View Area value, the area and range are displayed in the result window.

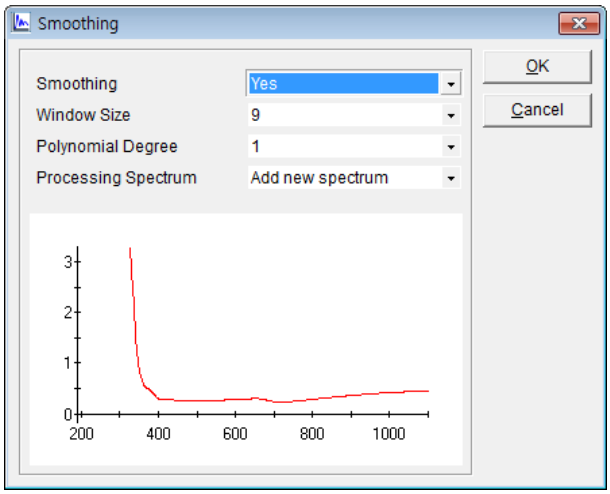




VII-2. Smoothing

- Use the Smoothing command to smooth the spectrum.
- Procedure

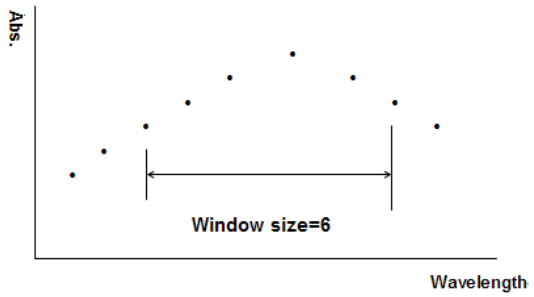
1. Select **Smoothing**. The following dialog box is displayed.



2. Set the function parameters.

   a. **Smoothing:** Select Yes or No.

   b. **Window Size**: Select the data point to use to smooth the spectrum.



c. **Polynomial Degree**: Select the dimension of curve fitting.

d. **Processing Spectrum**: Select Add new spectrum or Change original spectrum.

3. After setting parameters is complete, click **OK**. The result is displayed in the main window.

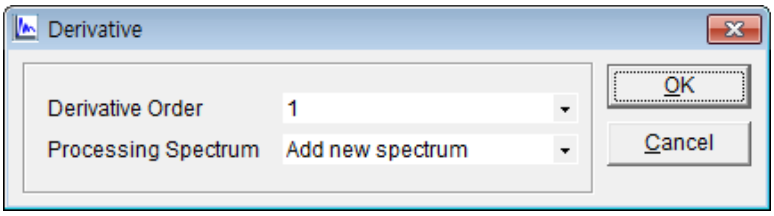
NOTE: ***Savitsky-Golay Smoothing***

UV Express uses the Savitsky-Golay method for the data smoothing. Using the Savitsky-Golay method results in the elimination of (window size)/2 points on each end of the smoothed value in the middle of the window. It is the preferred method for noise reduction and is also recommended for smoothing because no truncation of the data occurs.

VII-3. Derivative

- Use the Derivative command to obtain the derivative data of the spectrum.
- Procedure

1. Select **Derivative**. The following dialog box will be displayed.

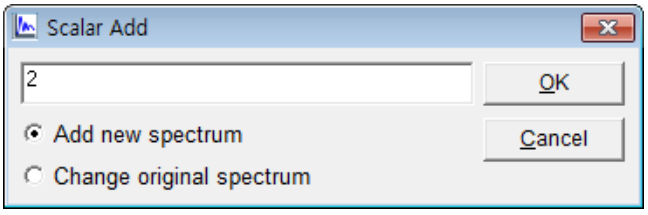


2. Select **OK** after entering the **Derivative Order** number and selecting the **Processing Spectrum**. The result will be displayed in the main window.

VII-4. Scalar Add

- Use the Scalar Add command to add a value to the Y-axis of a spectrum
- Procedure

1. Select **Scalar Add**. The following dialog box will be displayed.



2. Enter the value to add to the spectrum.

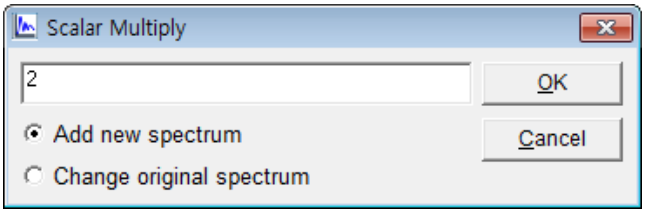
3. Select **Add new spectrum** or **Change original spectrum**.

4. Click **OK**. The result will be displayed in the main window.

VII-5. Scalar Multiply

- Use the Scalar Multiply command to multiply the Y-axis of a spectrum by a value
- Procedure

1. Select **Scalar Multiply**. The following dialog box will be displayed.



2. Enter the value to multiply the spectrum.

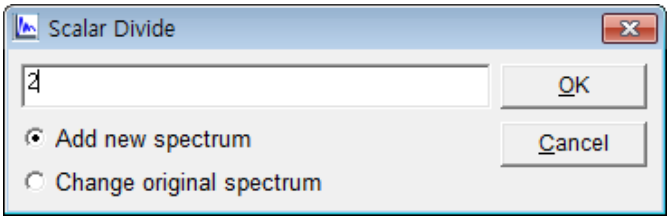
3. Select **Add new spectrum** or Change **original spectrum**.

4. Select **OK.** The result will be displayed in the main window.

VII-6. Scalar Divide

- Use the Scalar Divide command to divide the Y-axis of a spectrum by a value
- Procedure

1. Select **Scalar Divide**. The following dialog box will be displayed.



2. Enter the value to divide the spectrum.

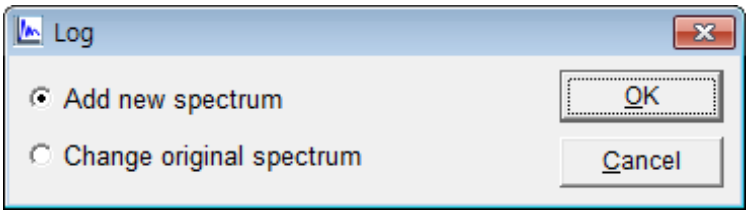
3. Select **Add new spectrum** or **Change original spectrum**.

4. Select **OK.** The result is displayed in the main window.

VII-7. Log

- Use the Log command to compute the log of a spectrum.
- Procedure

1. Select **Log**. The following dialog box will be displayed.



2. Select **Add new spectrum** or **Change original spectrum**.

3. Click **OK**. The result is displayed in the main window.

VII-8. Add

- Use the Add command to add the Y-axis values of selected spectra.
- Procedure

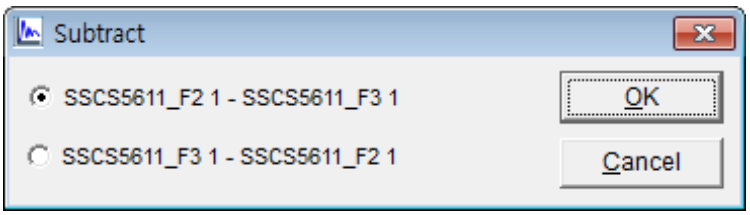
1. Select desired spectra to add by clicking the spectra while holding down the **Ctrl** key.

2. Select **OK**. The result is displayed in the main window.

VII-9. Subtract

- Use the Subtract command to subtract the Y-axis values of two spectra.
- Procedure

1. Select two spectra to subtract by clicking the spectra while holding down the **Ctrl** key. The following dialog box is displayed.



2. Select the appropriate equation.

3. Select **OK**. The subtracted result is displayed in the main window.

VII-10. Average

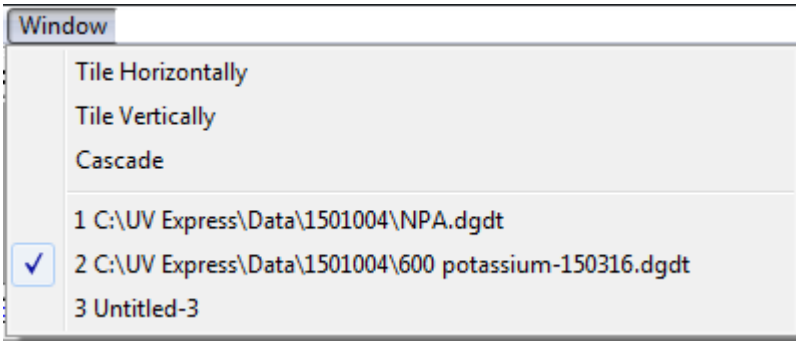
- Use the Average command to calculate the average of selected spectra.
- Procedure

1. Select the spectra to average together by clicking the spectra while holding down the **Ctrl** key or using the **Select All** command.

2. Select **OK**. The average result is displayed in the main window.

VIII. Window Menu

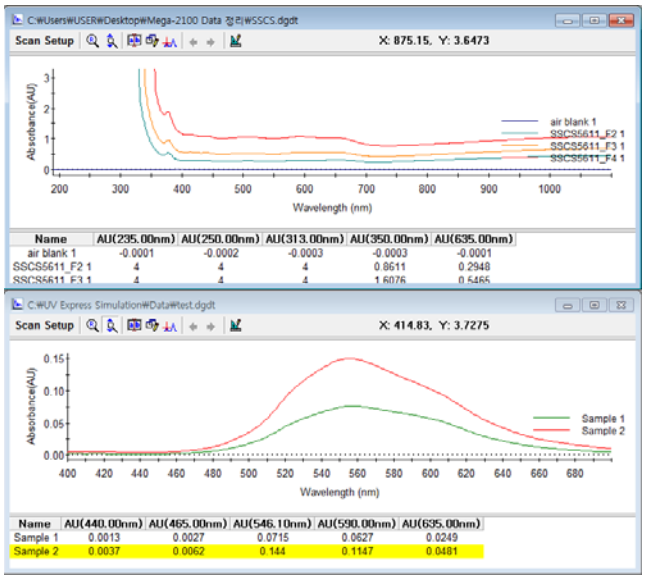
The Window Menu to arrange the windows and show the current windows.



Command	Functions
Tile Horizontally	Display Windows in horizontal the mode
Tile Vertically	Display Windows in vertical tile mode
Cascade	Display Windows in cascade mode

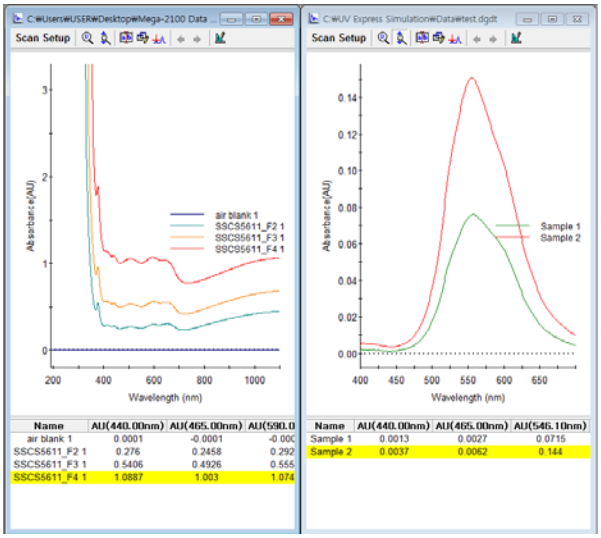
VIII-1. Tile Horizontally

Use the Tile Horizontally commands to align the windows in the horizontal tile modes as below.



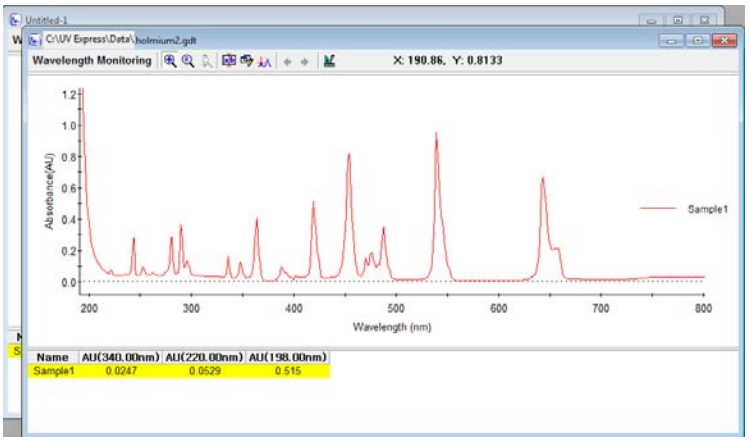
VIII-2. Tile Vertically

Use the Tile Vertically commands to align the windows in the vertical tile mode as below.

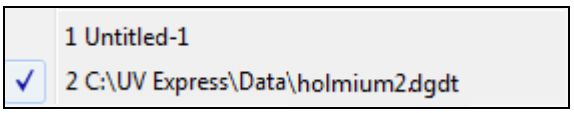


VIII-3. Cascade

Use the Cascade commands to align the windows in the cascade mode as below.



➢ The titles of the current windows are displayed as below.



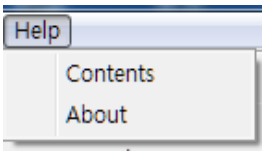
➢ Procedure

1. To view a different window, click the window you want to view and the selected window is displayed.

2. When the data in the window is saved, the file titles are listed.

IX. Help Menu

The Help Menu contains the Help contents for UV Express software.



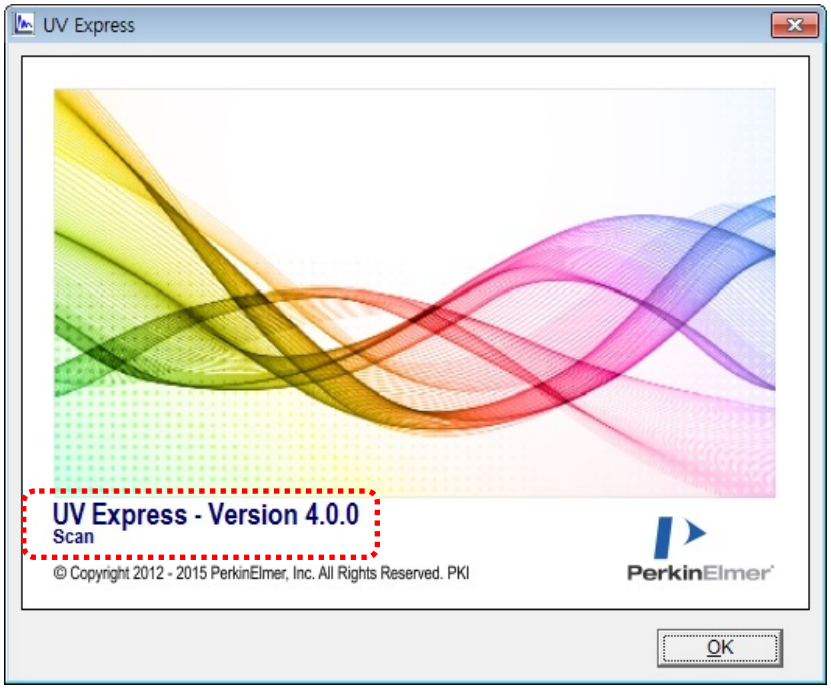
Command	Function
Contents	Open the UV Express Software Users Guide (Multi-Component Analysis Software Guide, in case of Multi-Component Analysis software is used)
About...	Display the version of UV Express Software

IX-1. Contents

Contents links to external document of the UV Express Software Users Guide. In the Multi-Component Analysis mode, Contents links to Multi-Component Analysis Software Guide.

IX-2. About

About UV Express contains information on the version of the software as shown below.



X. Measurement Modes

X-1. SCAN Mode

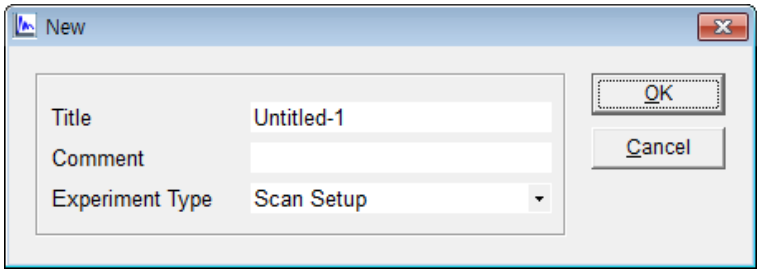
- This mode includes the following Experiment types:
  - Scan Setup
  - Equation Calculation
  - Find Peak/valley
- This SCAN mode is used to obtain the desired wavelength photometric values such as AU, %T and %R.
- Results in this mode are interchangeable. For example, data measured using Equation Calculation method can be opened in a Scan Setup method.
- Perform a General Method measurement as follows:
  1. Select measurement mode.
  2. Set method parameters.
  3. Collect the baseline.
  4. Measure samples.
  5. Save or print results as required.

X-1-1. Scan Setup

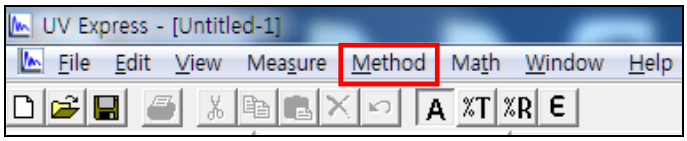
- Use Scan Setup to collect data over the full spectral range of the instrument, or a specified interval. Perform this type of experiment.
- Procedure

1. Execute the **Scan** .

2. The following window will be displayed. Enter Title, Comment and select Experiment Type and then, select **OK**.

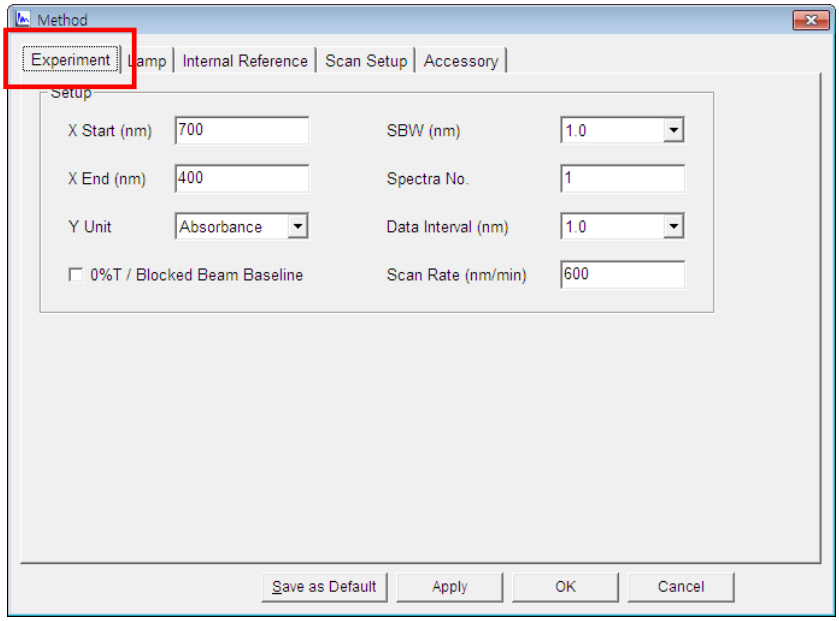


3. Select **Method**.



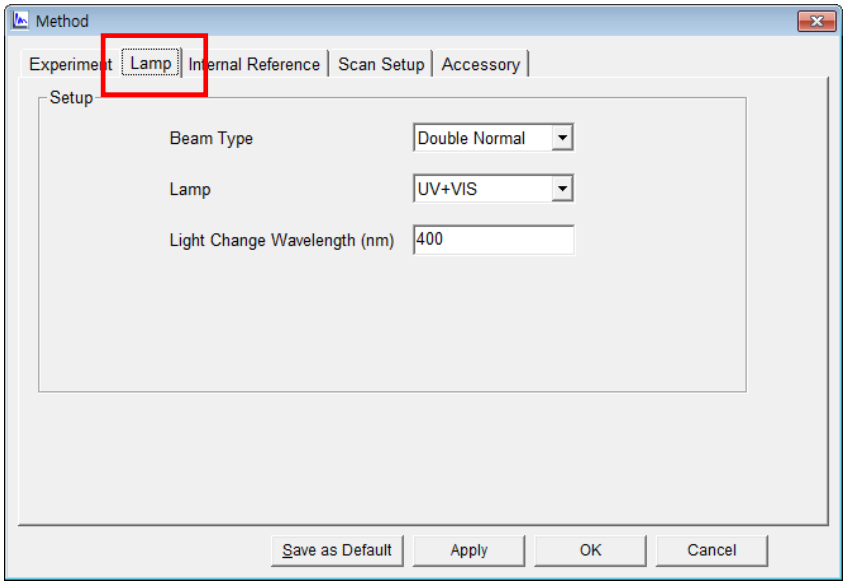
4. Setup the experiment parameters as follows:

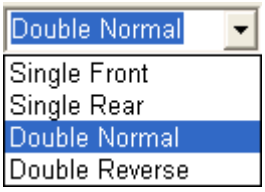
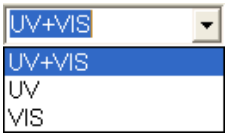
4.1 Experiment



Command	Function
X Start (nm)	Input the start measurement wavelength (190 to 1100 nm)
X End (nm)	Inputs the end measurement wavelength
Y Unit	Displays Y-axis unit: Absorbance (AU), Transmittance (%T), Reflectance (%R) or Energy
0%T / Blocked Beam Baseline	Check the **0%T/Blocked Beam Baseline** checkbox when measuring samples with high absorbance/low transmittance.
SBW	Select Spectral Band Width: 0.5, 1, 2, 5, 20 nm. Default is 1.0 nm.
Spectra No.	Parameter determines how many times the sample is tested (1 to 999)
Data Interval (nm)	Select Data Interval: 0.05, 0.1, 0.5, 1.0, 2.0 nm. Default is 1.0 nm.
Scan Rate (nm/min)	Shows the scan speed per time

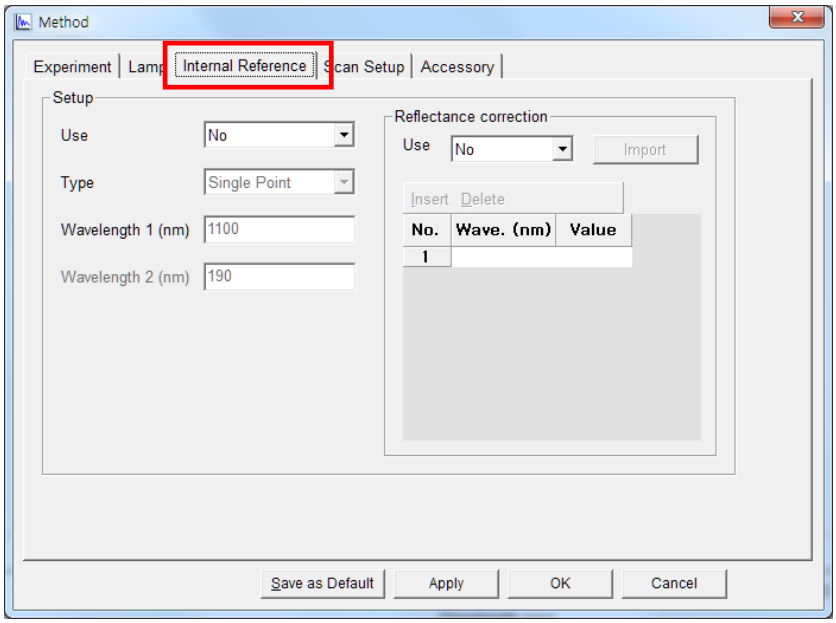
4.2 Lamp

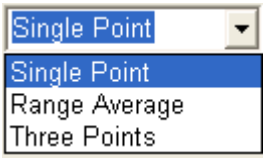


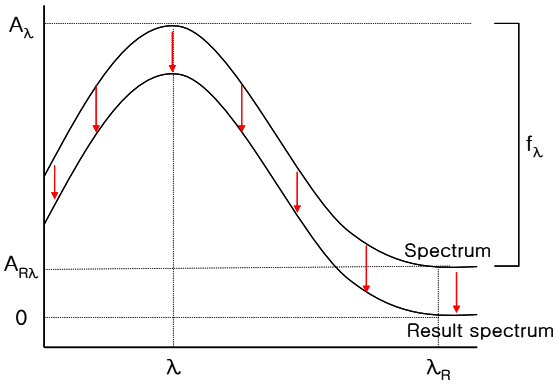
Command	Function
Beam Type	Select Beam Path Type  Single Front: Only uses Sample holder as a single beam type Single Rear: Only uses Reference holder as a single beam type Double Normal: General uses as a double beam type Double Reverse: Reverses the sample and reference position as a double beam type
Lamp	Select Lamp.  Light Change Wavelength (nm) will be deactivated if UV or Vis lamp is selected.
Light Change Wavelength (nm)	Sets the changeover wavelength for the deuterium lamp and halogen lamp. Enter a wavelength. (360~450 nm, default setting: 400 nm)

4.3 Internal Reference

Internal Reference is a technique that can be used to improve the precision of results by minimizing the effects of any changes that cause a linear baseline shift, for example a drift in lamp intensity. This technique is particularly useful for samples with low absorbance.



Command	Function
Use	Select using Internal Reference as Yes or No.
Type	Select Internal Reference type. There are three methods of calculating the baseline values. The value is calculated by method suited to each condition.  ***Single Point***: Single point method is used when the baseline shift is the same at all wavelengths. The reference wavelength is usually selected at a point on the baseline. Baseline value is eliminated by subtracting the absorbance at reference wavelength from the absorbance of full wavelength.



$$f_\lambda = A_\lambda - A_{R\lambda}$$

f_λ is the function result at wavelength λ

A_λ is the absorbance at wavelength λ

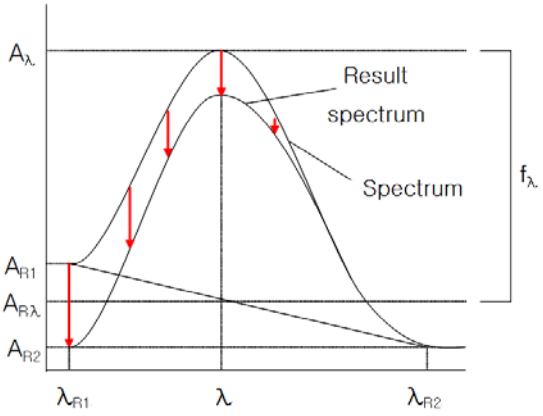
$A_{R\lambda}$ is the absorbance at reference wavelength λ_R

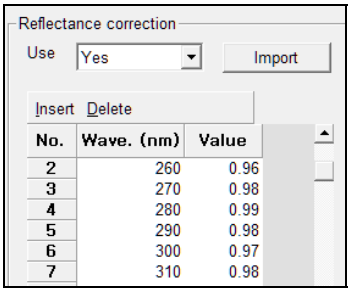
Range Average:

The range average method is as an extension of the single point method, is used when it is hard to select reference wavelength as a point. In this method, reference wavelength replaces the single wavelength absorbance value with the average absorbance value over a wavelength range.

Three Points:

Three points method is used correction of slant baseline, then the absorbance values from the two reference wavelengths, A_{R1} and A_{R2}, define a straight line which is used to calculate the reference absorbance ($A_{R\lambda}$) at the wavelength(λ). Result spectrum calculated by following equation.



	$$A_{R\lambda} = \frac{1}{\lambda_{R2} - \lambda_{R1}} \{(\lambda_{R2} - \lambda)A_{R1} + (\lambda - \lambda_{R1})A_{R2}\}$$ $$f_\lambda = A_\lambda - A_{R\lambda}$$ f_λ is the function result at wavelength λ A_λ is the absorbance at wavelength λ $A_{R\lambda}$ is the reference absorbance at wavelength R_λ
Wavelength 1 (nm)	Select the reference wavelength.
Wavelength 2 (nm)	Select the reference wavelength and this would be deactivated at Single Point type.
Reflectance correction	Reflectance correction allows user to collect absolute reflectance data. $R_{absolute} = F_{correction}\ R_{measured}$ $R_{absolute}$ is absolute specular reflection (%) $F_{correction}$ is the correction factor to correct the actual reflectance of standard mirror. $R_{measured}$ is actual measured specular reflection (%) Before calculating reflectance data, user must retrieve a standard reference file by clicking the **Import** button. This file must be obtained from the manufacturer of the standard [The imported file should be formatted in .CSV]. User can also manually enter the standard value using the **Insert** or **Delete** buttons. 1. Select **Yes.** 2. Enter the wavelengths and correction factors. 

3. If you have a standard reference file as CSV format, click **Import** and select the standard reference file to import rather than entering wavelengths and correction factors one by one.

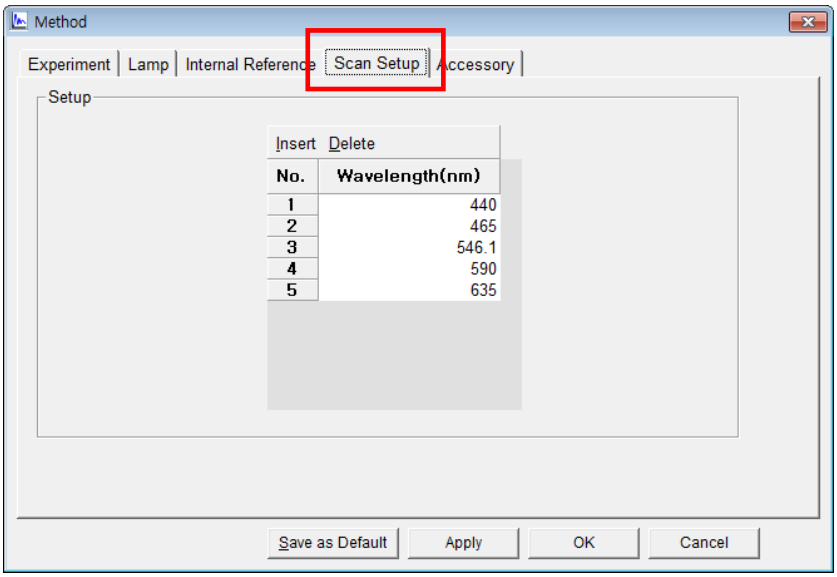
NOTE: *The data in an imported standard reference file (.CSV) should be formatted as shown in the picture below: wavelength-semicolon-factor.*

	A
1	250;0.9481
2	300;0.9746
3	350;0.9736
4	400;0.979
5	450;0.9812
6	500;0.9824
7	550;0.983
8	600;0.9836
9	650;0.9835
10	700;0.9837
11	750;0.9825
12	

4. Click **Apply** and then **OK**. The corrected spectrum and results are shown in the main result window.

4.4 Scan Setup

Enter the wavelength using **Insert** and **Delete**.



4.5 Accessory

For more details of accessory setting refer to each accessory manual.

5. After completing parameter setup for Experiment, Lamp, Internal Reference and Scan Setup, select **Apply** and then click **OK**.

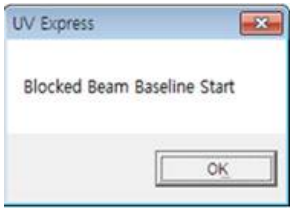
6. Depending on the samples, empty the cell holder or input the blank into Reference and Sample holder both. Close the sample compartment cover and measure Baseline using **Baseline** icon.

NOTE: *The baseline defines the absorbance 0 (or transmittance 100%) and is subtracted from the measurement result to give a correct sample spectrum (or is divided in the case of transmittance)*

NOTE: *When you check **the 0%T / Blocked Beam Baseline**, Baseline measurement procedure is as follows.*

*a. Click **Baseline** icon.*

*b. When the below message is popped up, click **OK** after checking the empty of both holders (Reference and Sample).*

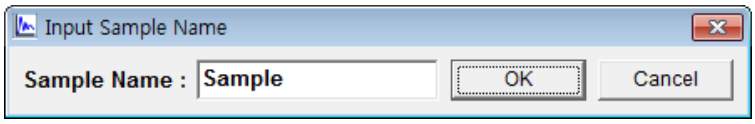


*c. Measure the dark automatically and then, after the below message is shown. Depending on the sample, empty the cell holder or input the blank into reference and sample holder both. Click **OK**.*

NOTE: *You should measure Baseline whenever the wavelength is changed or SBW is changed or Reference sample is changed.*

7. Input the sample into the sample holder and close the sample compartment cover and then, click **Sample** icon.

8. Input the sample name and click **OK**.



NOTE: *After finishing the measurement, if you want to change a sample name, select the sample name to be changed in the Result Window and press the Enter key after changing the name.*

9. The spectrum and result will be shown.

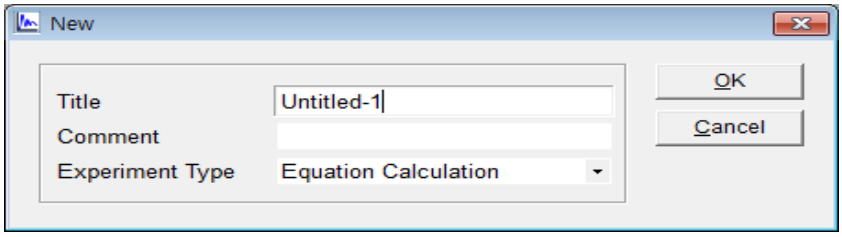
10. Save or print spectrum and results as desired.

X-1-2. Equation Calculation

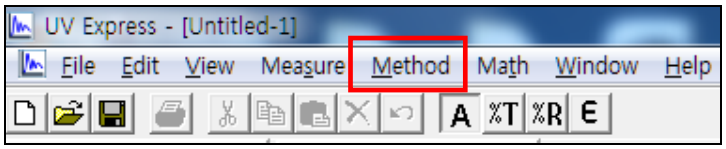
- Use Equation Calculation to collect data for a calculated result using a specified equation.
- Procedure

1. Execute the **Scan.**

2. The following window will be displayed. Enter Title, Comment and select Experiment Type and then, select **OK**.

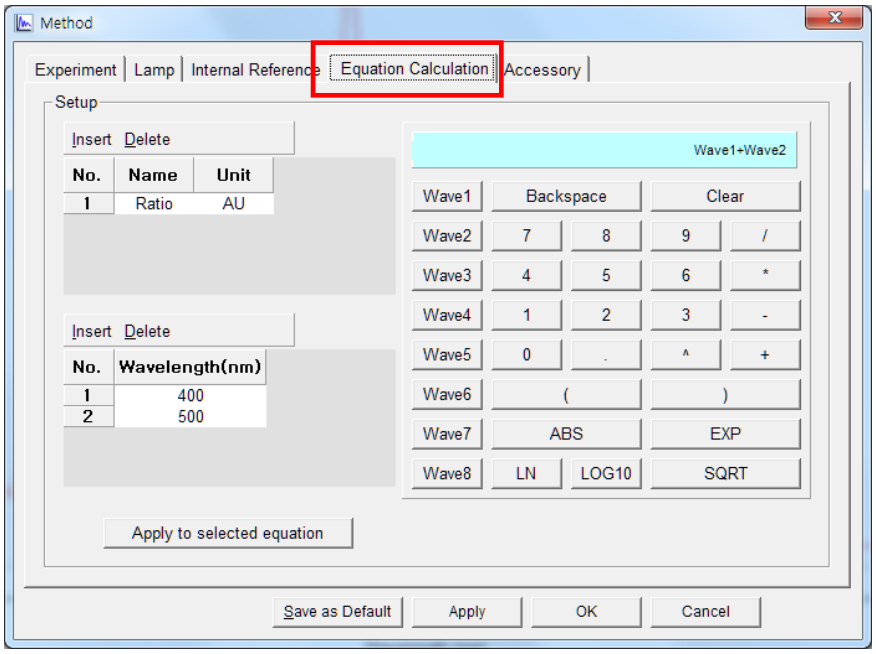


3. Select **Method**.



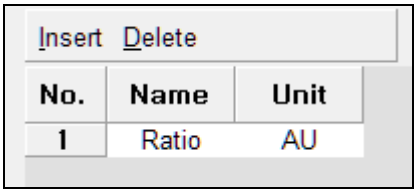
4. Setup the Experiment, Lamp and Internal Reference parameters. See **X-1-1. Scan Setup** for more details.

4.1 Equation Calculation

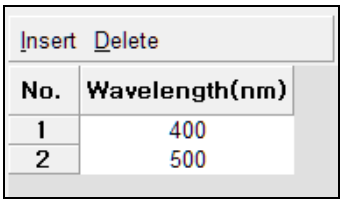


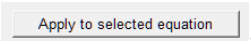
Command	Function
Setup	Set up a new equation and create a new row using Insert or Delete
Name	Enter the equation name
Unit	Enter the units of the Y-axis. Absorbance, transmittance or reflectance
Wavelength (nm)	Enter the wavelength to be used in the calculation. Up to 8 wavelengths (absorbance/transmittance value) can be input to the equation
ABS	Calculates the absolute value of the expression
EXP	Calculates exponential
LN	Calculates the natural logarithm of the expression
LOG10	Calculates the common logarithm of the expression
SQRT	Calculates the square root of the expression

5. Enter the Name and Unit of equation.

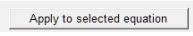


6. Enter the Wavelengths to make equation. User can add or remove the wavelength by clicking Insert or Delete button.

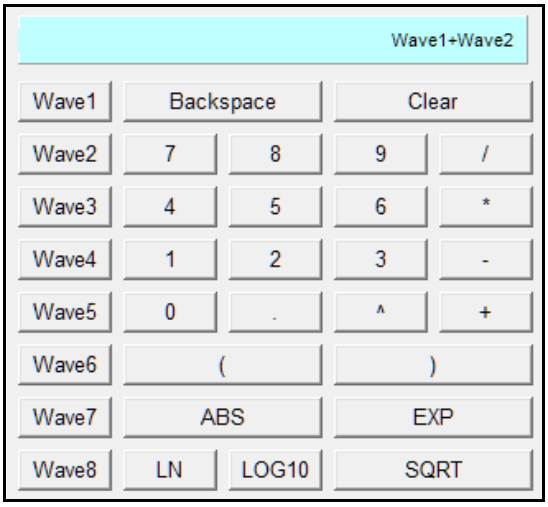


7. After completing equation, click **Apply to selected equation** 

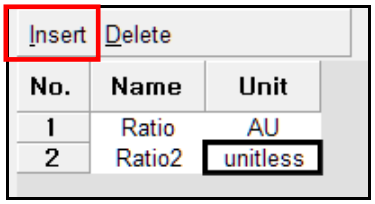
.

NOTE: Make *sure that whenever making each equation, user should always click the* ***Apply to selected equation*** 

button.



8. If you want to make another equation, add the Name and Unit of equation by clicking the **Insert** button.



9. Repeat procedure #6~7.

10. After completing parameter setup for Experiment, Lamp, Internal Reference and Equation Calculation, select **Apply** and then select **OK**.

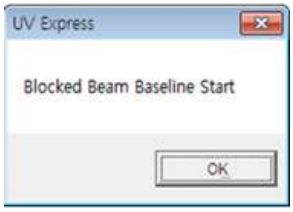
11. Depending on the samples, empty the cell holder or input the blank into Reference and Sample holder both. Close the sample compartment cover and measure Baseline using **Baseline** icon.

NOTE: *The baseline defines the absorbance 0(or transmittance 100%) and is subtracted from the measurement result to give a correct sample spectrum (or is divided in the case of transmittance)*

NOTE: *When you check **the 0%T / Blocked Beam Baseline**, Baseline measurement procedure is as follows.*

a. *Click **Baseline*** *icon.*

b. *When the below message is popped up, click **OK** after checking the empty of both holders (Reference and Sample).*

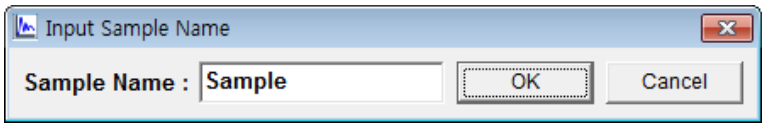


c. *Measure the dark automatically and then, after the below message is shown. Depending on the sample, empty the cell holder or input the blank into reference and sample holder both. Click **OK**.*

NOTE: *You should measure Baseline whenever the wavelength, SBW or Reference sample is changed.*

12. Input the sample into the sample holder and close the sample compartment cover and then, click **Sample** icon.

13. Input the sample name and click **OK**.



NOTE: *When you want to change the sample name, you can select [Name] in the Result Window (changed as yellow color) and should click Enter after changing the name.*

14. The spectrum and result will be shown.

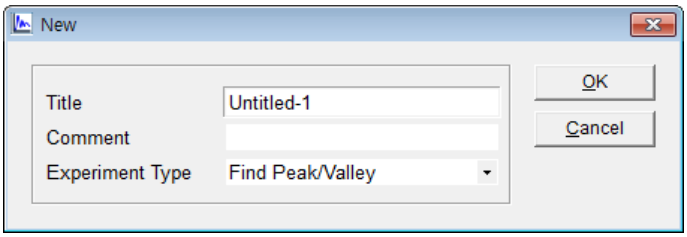
15. Save or print spectrum and results as desired.

X-1-3. Find Peak/Valley

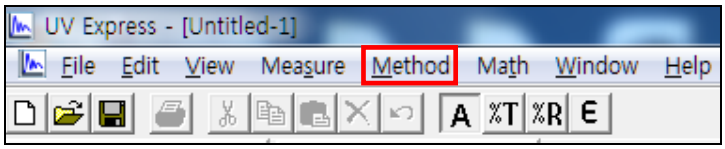
- Use **Find Peak/Valley** to determine the maxima and minima of Y-values in the defined wavelength range of the spectrum.
- Procedure

1. Execute the **Scan.**

2. The following window will be displayed. Enter Title, Comment and select Experiment Type and then, select **OK**.



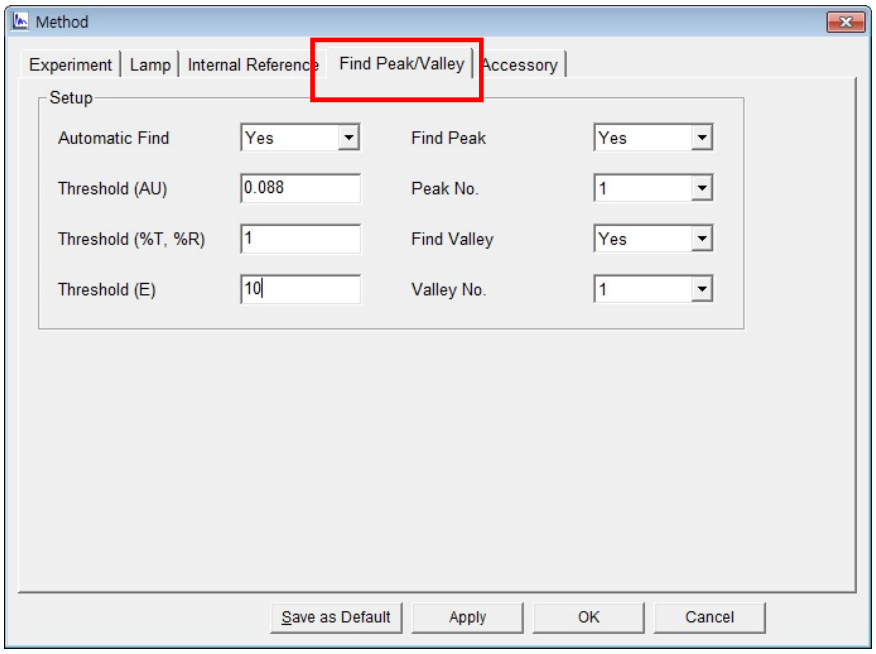
3. Click **Method**.



4. Setup the experiment, Lamp and Internal Reference parameters. See **X-1-1. Scan Setup** for more details.

NOTE: *You should enter the suitable sensitivity value and masking range to find the right peaks and valleys.*

4.1 Find Peak/Valley



Command	Function
Automatic Find	YES: Finds automatically NO: Finds manually
Threshold (AU)	Enter an absorbance value for the threshold. Peaks about this threshold are included in the result window.
Threshold (%T, %R)	Enter a transmittance (or reflectance) value for the threshold. Peaks about this threshold are included in the result window.
Threshold (E)	Enter an intensity value for the threshold. Peaks about this threshold are included in the result window.
Find Peak	Select Yes or No for finding peaks.
Peak No.	Enter the numbers of peak to find. The number of peak can be selected from 1 to 39 or all of the peaks.
Find Valley	Select Yes or No for finding valleys.
Valley No.	Enter the numbers of valley to find. The number of valley can be selected from 1 to 39 or all of the valleys.

5. After completing parameter setup for Experiment, Lamp, Internal Reference and Find Peak/Valley, click **Apply** and then click **OK**.

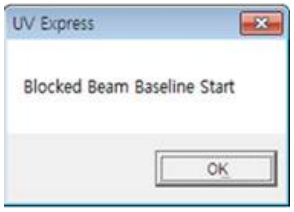
6. Depending on the samples, empty the cell holder or input the blank into Reference and Sample holder both. Close the sample compartment cover and measure Baseline using **Baseline** icon.

NOTE: *The baseline defines the absorbance 0(or transmittance 100%) and is subtracted from the measurement result to give a correct sample spectrum (or is divided in the case of transmittance).*

NOTE: *When you check **the 0%T / Blocked Beam Baseline**, Baseline measurement procedure is as follows.*

*a. Click **Baseline** icon.*

*b. When the below message is popped up, click **OK** after checking the empty of both holders (Reference and Sample).*

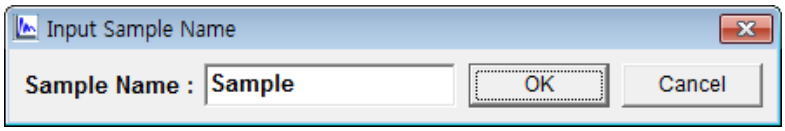


*c. Measure the dark automatically and then, after the below message is shown. Depending on the sample, empty the cell holder or input the blank into reference and sample holder both. Click **OK**.*

NOTE: *You should measure Baseline whenever the wavelength, SBW or Reference sample is changed.*

7. Input the sample into the sample holder and close the sample compartment cover and then, select **Sample** icon.

8. Input the sample name and select **OK**.



NOTE: *When you want to change the sample name, you can select [Name] in the Result Window (changed as yellow color) and should click Enter after changing the name.*

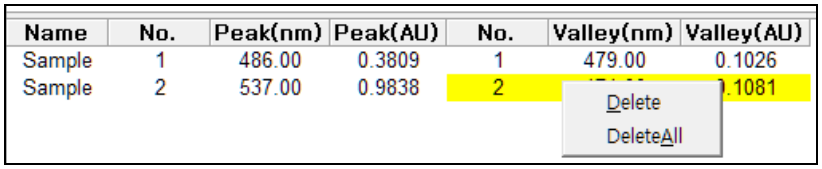
9. The spectrum and result will be displayed. Peaks and valleys are found automatically.

10. For manual peak/valley finding, use the following icons on Spectral Display Function Tool after set Automatic Find as **No**.

    Pick Peak Pick Valley

    After selecting the icon, you can check the peak/valley using the mouse-drag.

11. If you want to remove the peak/valley value, select it (the color will be chanced as yellow) and click the right button of mouse and select the command.



12. Save or print spectrum and results as desired.

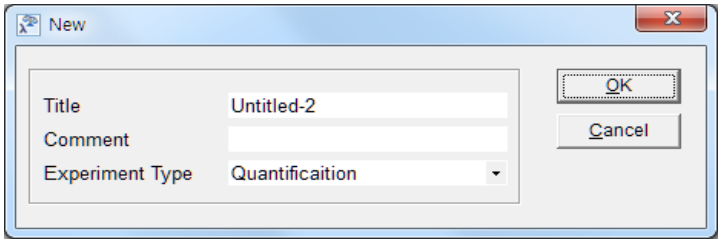
X-2. Quantification Mode

- Quantification Mode measures the unknown samples' concentration based on the obtained standards calibration curve.
- Use Quantification mode to quantify a sample at a single wavelength using a reference standard.
- Perform a Quantification Method measurement as follows:
  1. Select measurement mode.
  2. Set method parameters.
  3. Measure Baseline.
  4. Measure standards. Create the calibration curve using a set of standards.
  5. Measure samples.
  6. Save or print results as required.

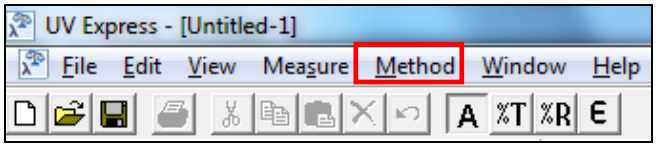
- Procedure

1. Execute the **Quantification** .

2. The following window will be displayed. Enter Title, Comment and select Experiment Type and then, click **OK**.

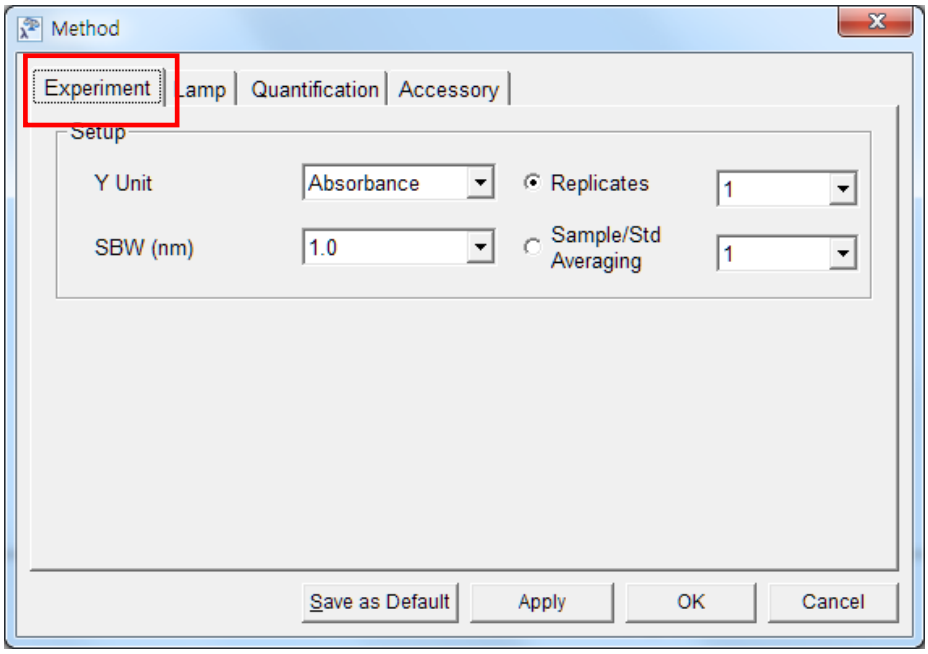


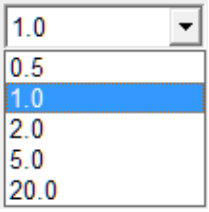
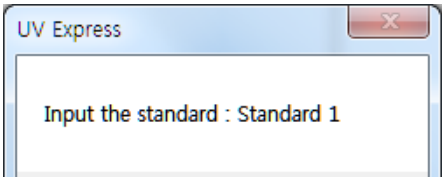
3. Select **Method**.



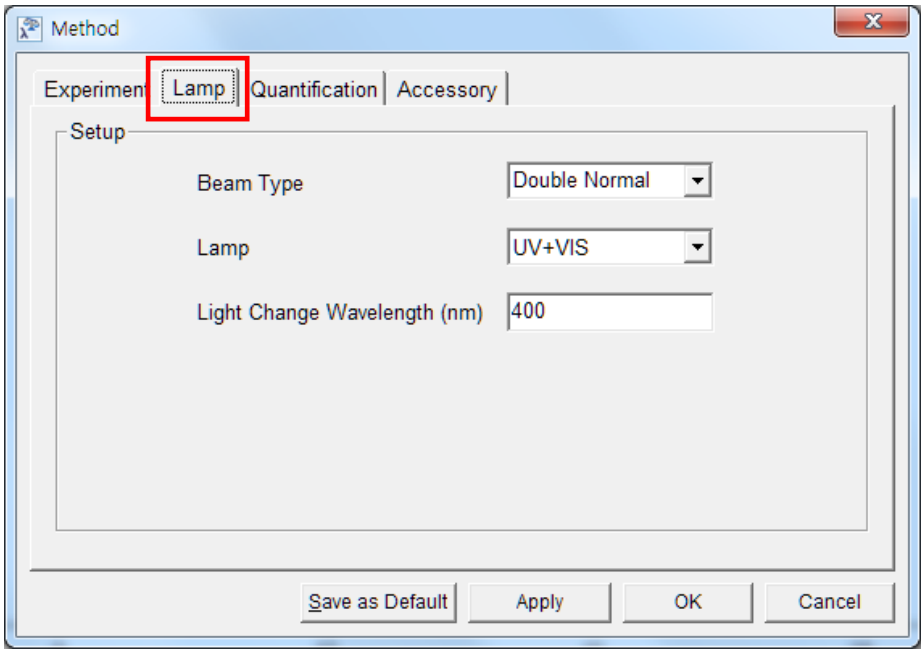
4. Setup the Experiment, Lamp and Quantification parameters.

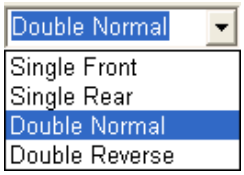
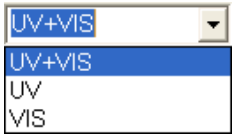
4.1 Experiment



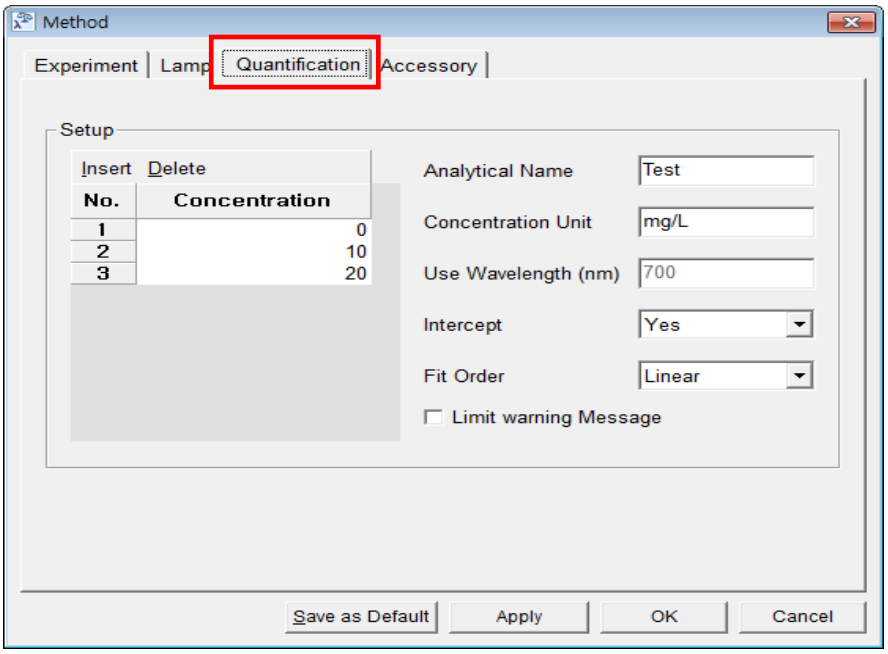
Command	Function
Y Unit	Displays Y-axis unit: Absorbance (AU), Transmittance (%T), Reflectance (%R) or Energy
SBW	Select Spectral Band Width (Default: 1.0) 
Replicates	Select the number of replicate readings to be taken for each sample (1~5). The measurement is automatically repeated as set number.
Sample/Std Average	Select the number of reading to be taken from different solutions of sample/standards (1~3). Sample/Std has to be replaced between each count. 

4.2 Lamp



Command	Function
Beam Type	Select Beam Path Type  Single Front: Only uses Sample holder as a single beam type Single Rear: Only uses Reference holder as a single beam type Double Normal: General uses as a double beam type Double Reverse: Reverses the sample and reference position as a double beam type
Lamp	Select Lamp  Light Change Wavelength(nm) will be deactivated if UV or VIS lamp is selected
Light Change Wavelength (nm)	Sets the changeover wavelength for the deuterium lamp and halogen lamp. Enter a wavelength (360 ~ 450 nm, default setting: 400 nm)

4.3 Quantification



Command	Function
Setup	Enter each standard concentration using Insert and Delete.
Analytical Name	Enter the file name
Concentration Unit	Enter the unit of concentration
Use Wavelength (nm)	Enter the wavelength to test
Intercept	No: Calibration curve passes through the origin Yes: Calibration curve does not pass through the origin
Fit Order	Choose the calibration curve dimension Linear, Quadratic or Cubic
Limit Warning Message	When the sample measurement value is out of the calibration curve range, the warning message will be shown. It can be selected as an option.

4.4 Accessory

For more details of accessory setting refer to each accessory manual.

5. After completing parameter setup for Experiment, Lamp and Quantification, select **Apply** and then select **OK**.

NOTE: *You can save the method and open the method whenever you need.*

ex) Save [File] → [Save Method] and Open [File] → [Open Method]

6. Input the blank solution into Reference and Sample holder both. Close the sample compartment cover and measure Baseline using **Baseline**

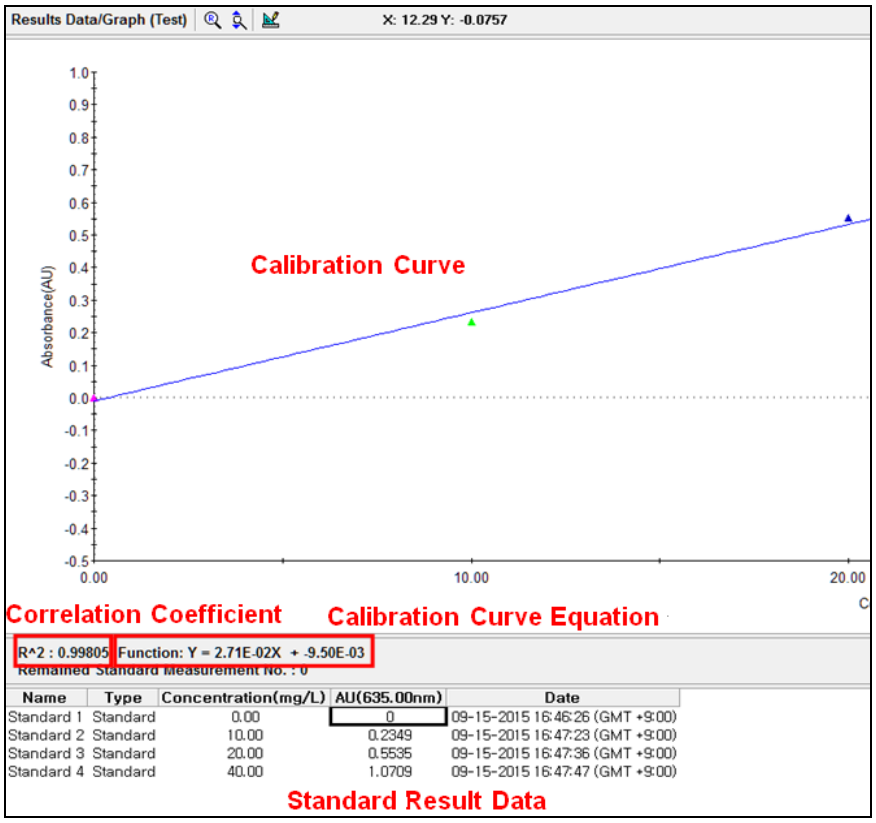
icon. Absorbance of selected wavelength is changed about 0 AU.

NOTE: *You should measure Baseline whenever the wavelength, SBW or Reference sample is changed.*

7. Input the Standard sample into the Sample holder and then, select the **Standard**

.

8. Measure Standard samples in order and the calibration curve will be created as follow.



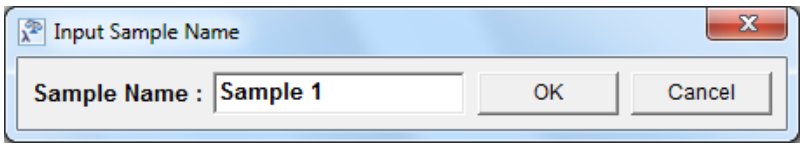
NOTE: *You can save the standard calibration curve and open the curve whenever you need.*

9. Input Blank and Unknown sample into each Reference and Sample holder and then, click **Sample**

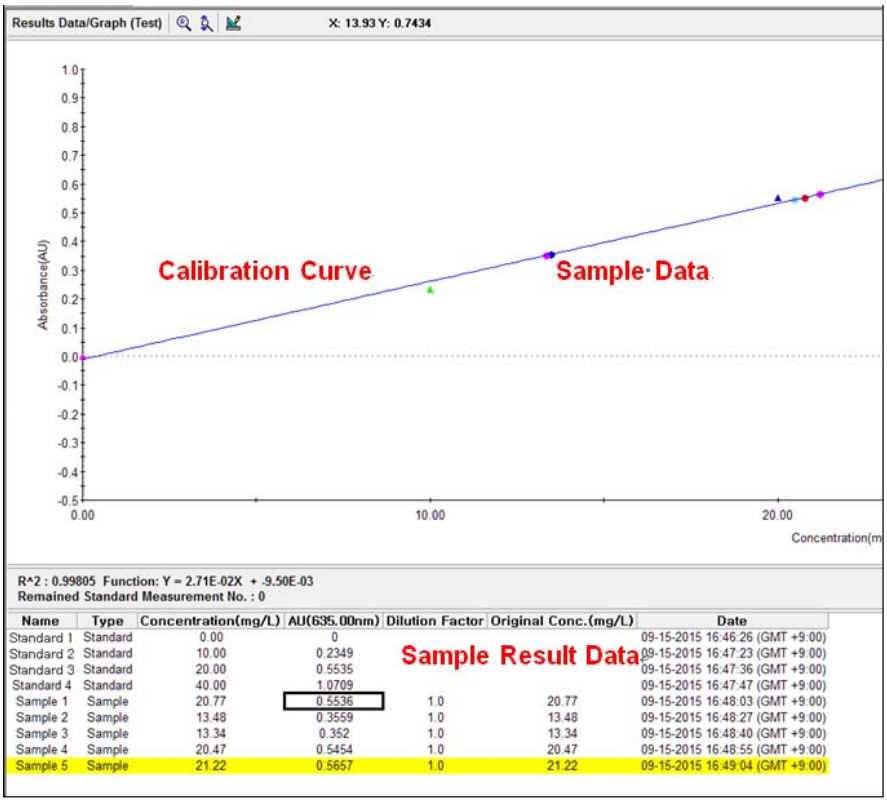
icon.

NOTE: *Click **Sample** after measuring [Baseline] when you opened the standard curve saved.*

10. Input sample name and select **OK**.



11. Data will be shown as follows.



Name	Type	Concentration(mg/L)	AU(635.00nm)	Dilution Factor	Original Conc.(mg/L)	Date
Standard 1	Standard	0.00	0			09-15-2015 16:46:26 (GMT +9:00)
Standard 2	Standard	10.00	0.2349			09-15-2015 16:47:23 (GMT +9:00)
Standard 3	Standard	20.00	0.5535			09-15-2015 16:47:36 (GMT +9:00)
Standard 4	Standard	40.00	1.0709			09-15-2015 16:47:47 (GMT +9:00)
Sample 1	Sample	20.77	0.5536	1.0	20.77	09-15-2015 16:48:03 (GMT +9:00)
Sample 2	Sample	13.48	0.3559	1.0	13.48	09-15-2015 16:48:27 (GMT +9:00)
Sample 3	Sample	13.34	0.352	1.0	13.34	09-15-2015 16:48:40 (GMT +9:00)
Sample 4	Sample	20.47	0.5454	1.0	20.47	09-15-2015 16:48:55 (GMT +9:00)
Sample 5	Sample	21.22	0.5657	1.0	21.22	09-15-2015 16:49:04 (GMT +9:00)

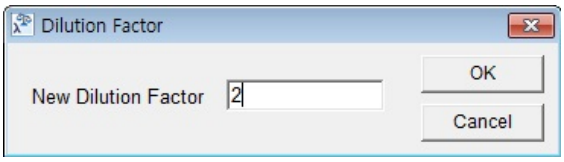
a. Name: Shows the entered Standard or Sample name.

b. Type: Displays the Standard or Sample.

c. Concentration: Display the calculated concentration.

d. AU: Displays the measured Y-axis value.

e. Dilution factor: The dilution factor is set 1.0 as a default and it can be edited after the sample measurement. Double-click on the Dilution Factor to be changed in each sample measurement result and enter a new Dilution Factor and click **OK**.

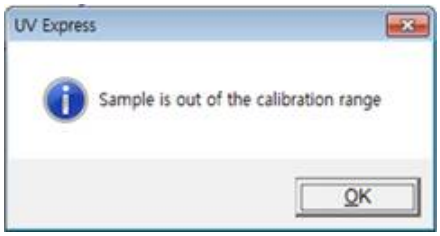


f. Original Conc.: Shows the original concentration of sample.

(Original Concentration = Concentration x Dilution Factor)

g. SD, %RSD: Shows the statistics of the repeated measures data.

NOTE: *In case of checking on Limit warning Message in the Quantification tab of the Method window, the following message is shown as the sample measurement values is out of the calibration curve range.*



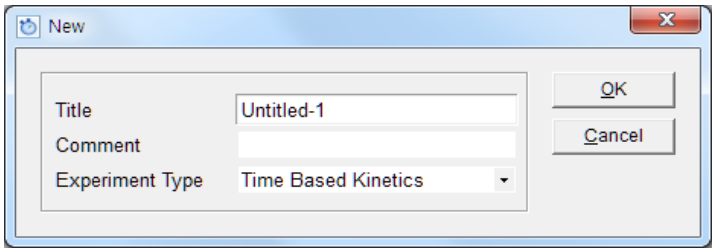
12. Save or print spectrum and results as desired.

X-3. Kinetics Mode

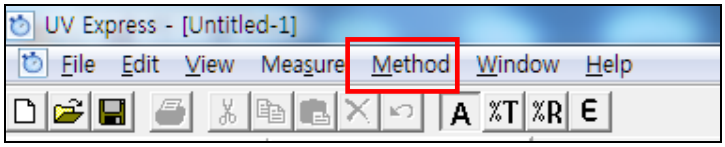
- Use Kinetics to test reaction rate. Kinetics mode measures the changes to a sample's absorbance, transmittance and reflectance over time at a fixed wavelength.
- Perform a Kinetics Method measurement as follows:
  1. Select measurement mode.
  2. Set method parameters.
  3. Measure Baseline.
  4. Measure samples.
  5. Save or print results as required.
- Procedure

1. Execute the **Kinetics** .

2. The following window will be displayed. Enter Title, Comment and select Experiment Type and then, select **OK**.

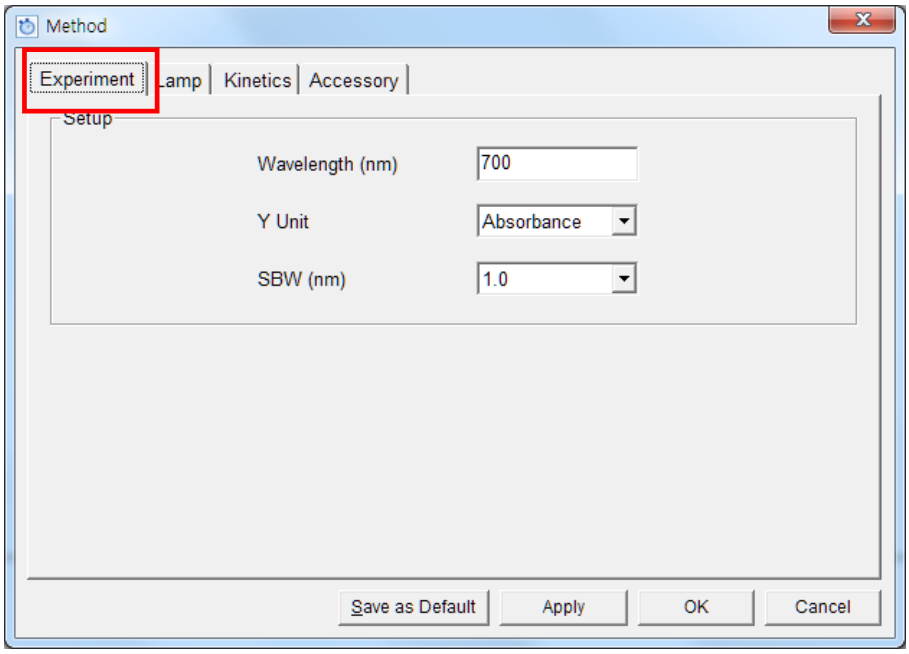


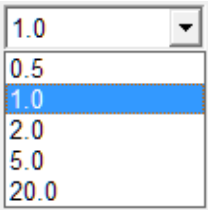
3. Select **Method**.



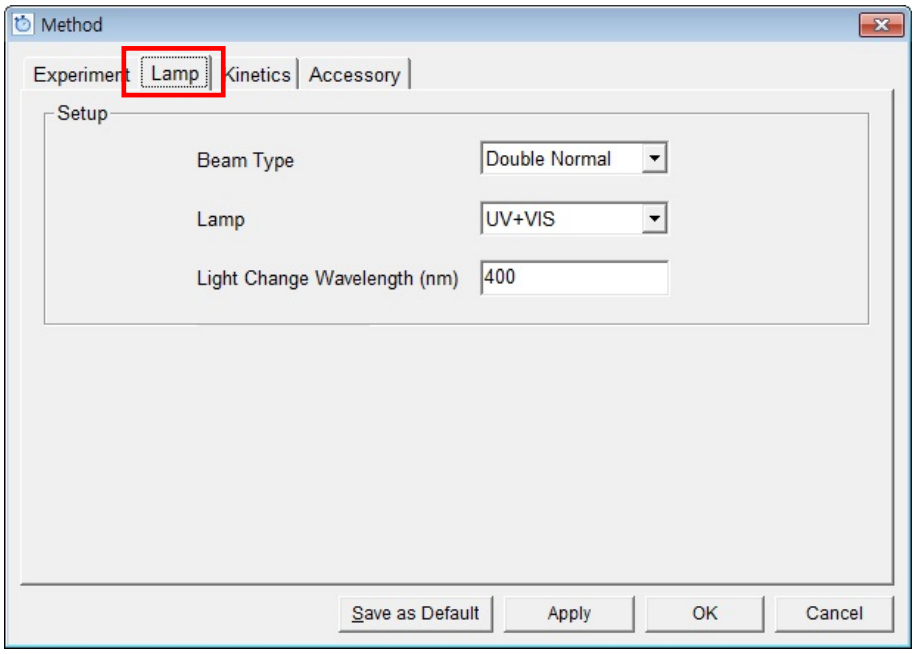
4. Setup the experiment, Lamp and Kinetics parameters.

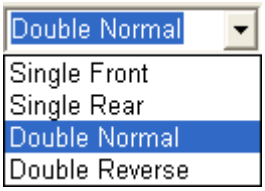
4.1 Experiment



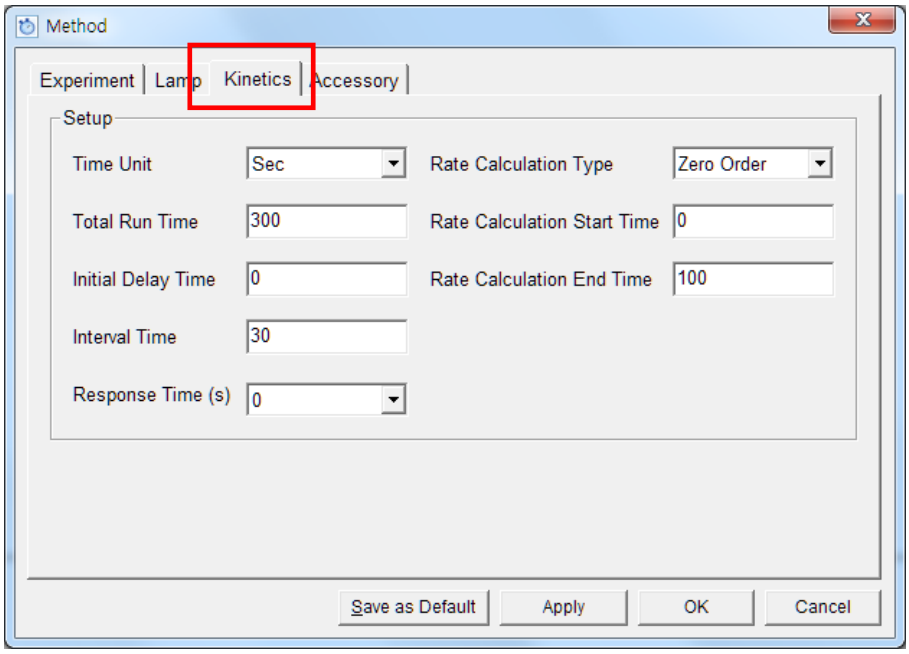
Command	Function
Wavelength (nm)	Enter a specific wavelength.
Y Unit	Displays Y-axis unit Absorbance: (AU) Transmittance: (%T) Reflectance: (%R) Energy
SBW (nm)	Select Spectral Band Width (Default: 1.0 nm) 

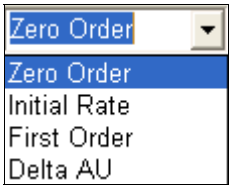
4.2 Lamp



Command	Function
Beam Type	Select Beam Path Type.  Single Front: Only uses Sample holder as a single beam type Single Rear: Only uses Reference holder as a single beam type Double Normal: General uses as a double beam type Double Reverse: Reverses the sample and reference position as a double beam type
Lamp	Select Lamp: UV+Vis, UV or VIS Light Change Wavelength (nm) will be deactivated if UV or VIS lamp is selected.
Light Change Wavelength (nm)	Sets the changeover wavelength for the deuterium lamp and halogen lamp. Enter a wavelength. (360 ~ 450 nm, default setting: 400 nm)

4.3 Kinetics



Command	Function
Time Unit	Choose a time unit either sec or min.
Total Run Time	Enter the total running time for testing samples.
Initial Delay Time	You can give a delay time before the real sample measurement. Ex) There is no stand-by time if you enter 0 for the initial delay time.
Interval Time	Enter the interval time between measurements.
Response Time	Select Response Time: 0, 0.1, 0.2, 0.5, 1, 2.5 or 10. It is the scanning time that stayed at a wavelength. Zero is the fastest scanning time that remained at a wavelength as short as possible.
Rate Calculation Type	Select Calculation Type  ▶ Zero Order The zero order calculation uses a linear fit to calculate the rate, k, b linear regression using the equation:

$A_t = A_0 + kt$

A_t is the absorbance at time **t**
A_0 is the absorbance at the start of the calculation time range
k is the zero order rate constant [Units: 1/ AU·s]

▶ **Initial Rate**

The initial rate calculation uses a quadratic fit to calculate the rate, k, by linear regression using the equation:

$A_t = A_0 + kt + t^2$

A_t is the absorbance at time **t**
A_0 is the initial absorbance
k is the initial rate [Units: AU/s]

First order

The first order calculation uses an exponential fit to calculate the rate, k, using:

$A_t = A_\infty + (A_o - A_\infty)\, e^{-kt}$

This rate law can be rearranged to

$\ln(A_t - A_\infty) = \ln(A_o - A_\infty) - kt$

A_t is the absorbance at time **t**
A_0 is the initial absorbance
k is the first order rate constant [Unit: 1/s]

▶ **Delta AU**

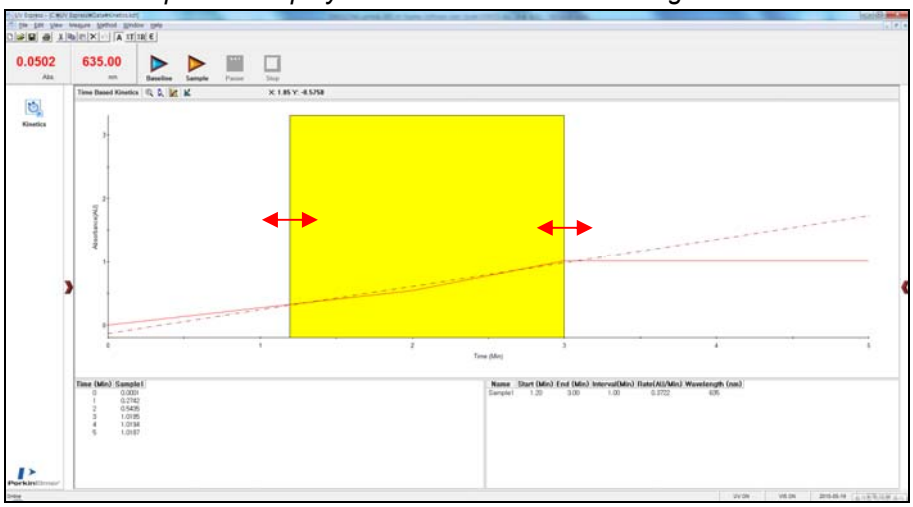
Delta AU is the difference between the absorbance at the start of the calculation time range and the absorbance at the end. The calculation is very simple:

Delta AU = At - A0

At is the absorbance at time **t**
A_0 is the initial absorbance
Delta AU [Units: AU]

Rate Calculation Start Time	Input the start time for Kinetic Rate calculation.
Rate Calculation End Time	Input the end time for Kinetic Rate calculation.

NOTE: *User can change the rate calculation range roughly by dragging the mouse. Select Cursor* *icon in Spectral Display Function Tool to set the range.*



4.4 Accessory

For more details of accessory setting refer to each accessory manual.

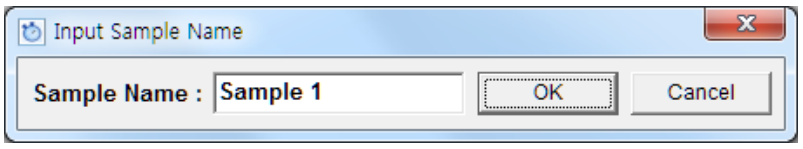
5. After completing parameter setup for Experiment, Lamp and Kinetics, select **Apply** and then select **OK**.

6. Input the blank solution into Reference and Sample holder both. Close the sample compartment cover and measure Baseline using **Baseline** icon. Absorbance of selected wavelength is changed about 0 AU.

NOTE: *You should measure Baseline whenever the wavelength, SBW or Reference sample is changed*

7. Input sample solution into the sample holder and then select **Sample** icon.

8. Input sample name and select **OK**.



9. If you want to stop for a while on the way of measurement, select **Pause** icon. Resume by selecting **Restart** icon.

10. After the experiment is completed, confirm the result data.

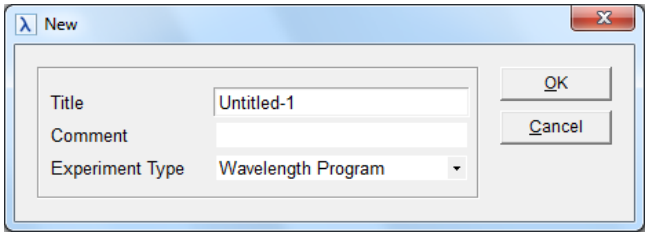
11. Save or print spectrum and results as desired.

X-4. Wavelength Program Mode

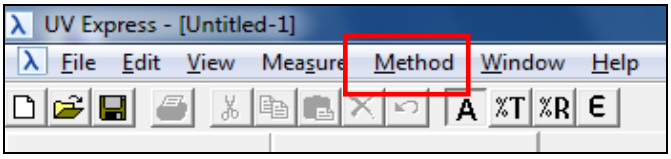
- Wavelength Program Mode measures the absorbance, Transmittance and reflectance at selected wavelengths.
- Perform a Wavelength Program Method measurement as follows:
  1. Select measurement mode.
  2. Set method parameters.
  3. Measure Baseline.
  4. Measure samples.
  5. Save or print results as required.
- Procedure

1. Execute the Wavelength Program .

2. The following window will be displayed. Enter Title, Comment and select Experiment Type and then, select **OK**.

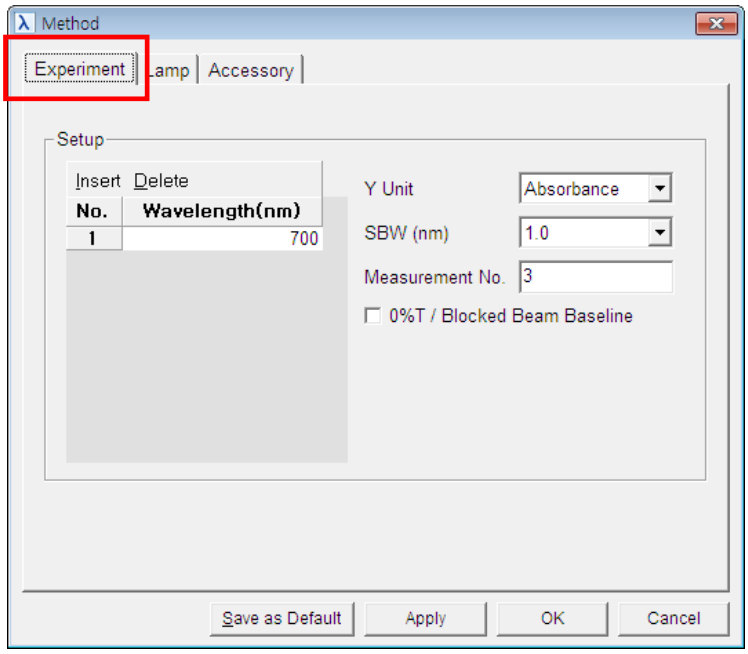


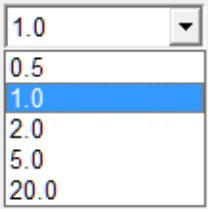
3. Select **Method**.



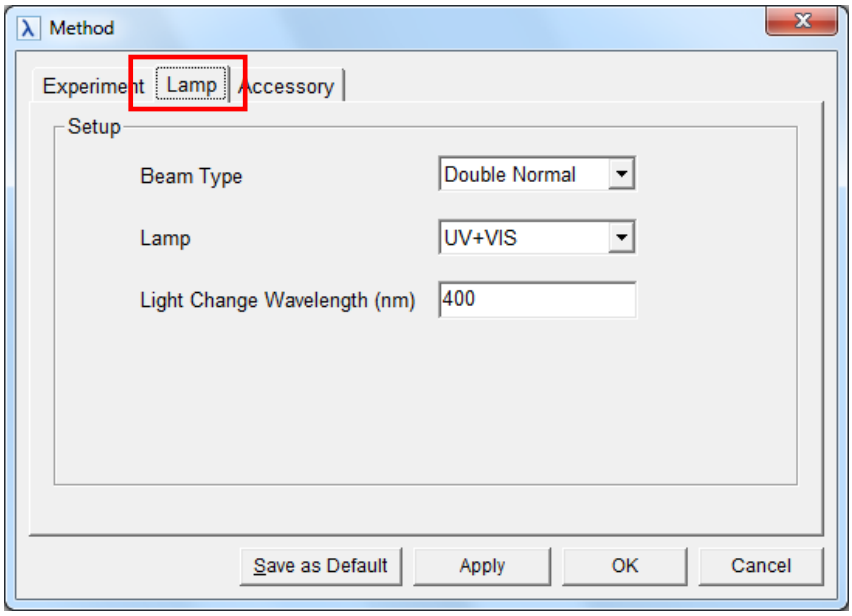
4. Setup the Experiment Setup and Lamp parameters.

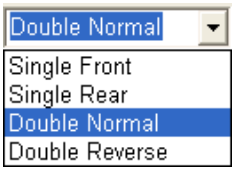
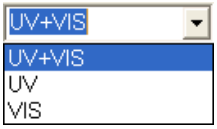
4.1 Experiment Setup



Command	Function
Wavelength (nm)	Enter a specific wavelength
Y Unit	Displays Y-axis unit Absorbance: (AU) Transmittance: (%T) Reflectance: (%R) Energy
SBW	Select Spectral Band Width (Default: 1.0) 
Measurement No.	Enter the number of repeated sample measurement.
0%T / Blocked Beam Baseline	Check the **0%T/Blocked Beam Baseline** checkbox when measuring samples with high absorbance/low transmittance.

4.2 Lamp



Command	Function
Beam Type	Select Beam Path Type  Single Front Single Rear Double Normal Double Reverse Single Front: Only uses Sample holder as a single beam type Single Rear: Only uses Reference holder as a single beam type Double Normal: General uses as a double beam type Double Reverse: Reverses the sample and reference position as a double beam type
Lamp	Select Lamp.  UV+VIS UV VIS Light Change Wavelength (nm) will be deactivated if UV or VIS lamp is selected.
Light Change Wavelength (nm)	Sets the changeover wavelength for the deuterium lamp and halogen lamp. Enter a wavelength. (360~450 nm, default setting: 400 nm)

4.3 Accessory

For more details of accessory setting refer to each accessory manual.

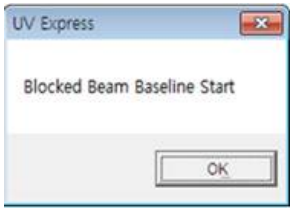
5. After completing parameter setup for Experiment Setup and Lamp, select **Apply** and then select **OK**.

6. Input the blank solution into Reference and Sample holder both. Close the sample compartment cover and measure Baseline using **Baseline** icon. Absorbance of selected wavelength is changed about 0 AU.

NOTE: *When you check **the 0%T / Blocked Beam Baseline**, Baseline measurement procedure is as follows.*

*a. Click **Baseline** icon.*

*b. When the below message is popped up, click **OK** after checking the empty of both holders (Reference and Sample).*

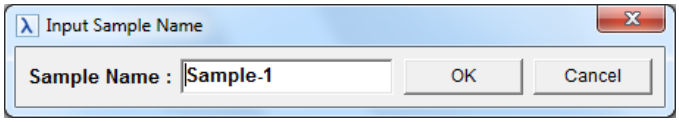


*c. Measure the dark automatically and then, after the below message is shown. Depending on the sample, empty the cell holder or input the blank into reference and sample holder both. Click **OK**.*

NOTE: *You should measure Baseline whenever the wavelength, SBW or Reference sample is changed.*

7. Input the sample into the sample holder and close the sample compartment cover and then, select **Sample** icon.

8. Input sample name and select **OK**.



9. Data will be displayed in the result window.

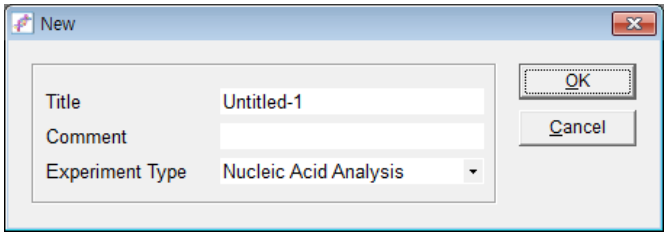
10. Save or print spectrum and results as desired.

X-5. Nucleic Acid Analysis Mode

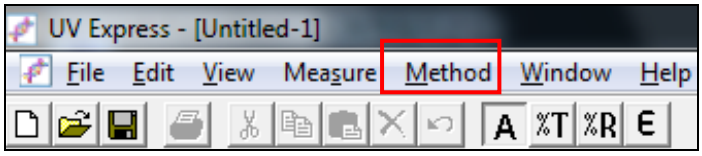
- Use Nucleic Acid Analysis Methods to perform ratio and concentration measurements on samples containing proteins and nucleic acids.
- Perform a Nucleic Acid Method measurement as follows:
  1. Execute Nucleic Acid Analysis mode.
  2. Set method parameters.
  3. Measure Baseline.
  4. Measure samples.
  5. Save or print results as required.
- Procedure

1. Execute the **Nucleic Acid Analysis** **.**

2. The following window will be displayed. Enter Title, Comment and select Experiment Type and then, select **OK**.

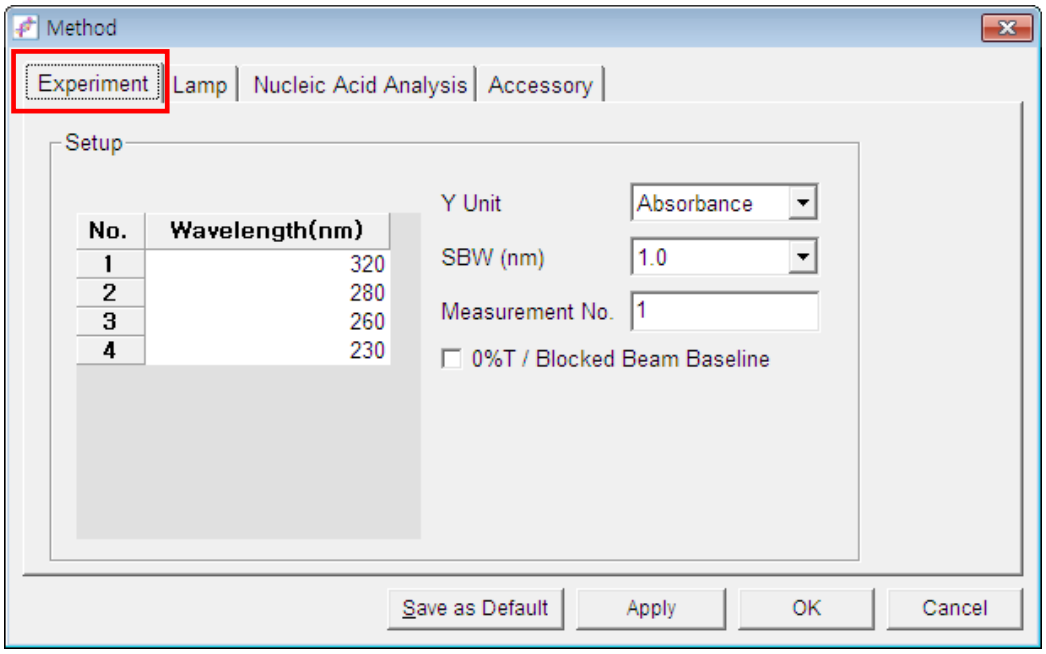


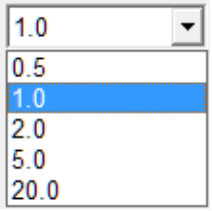
3. Select **Method**.



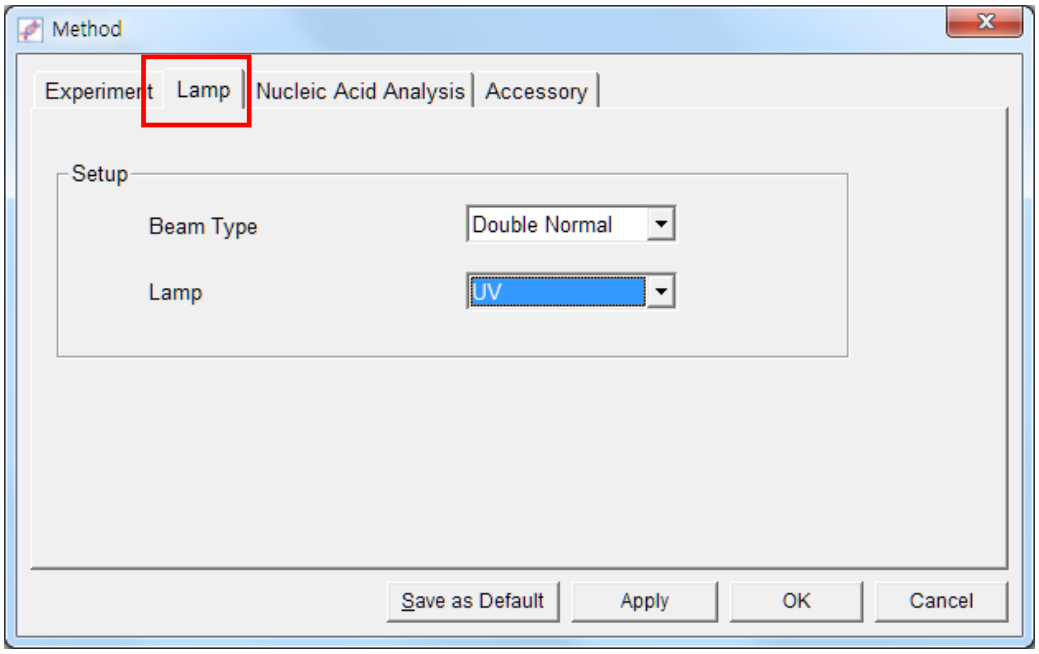
4. Setup the Experiment Setup and Lamp parameters.

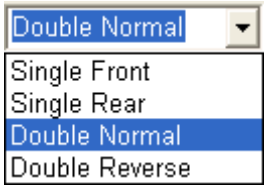
4.1 Experiment Setup



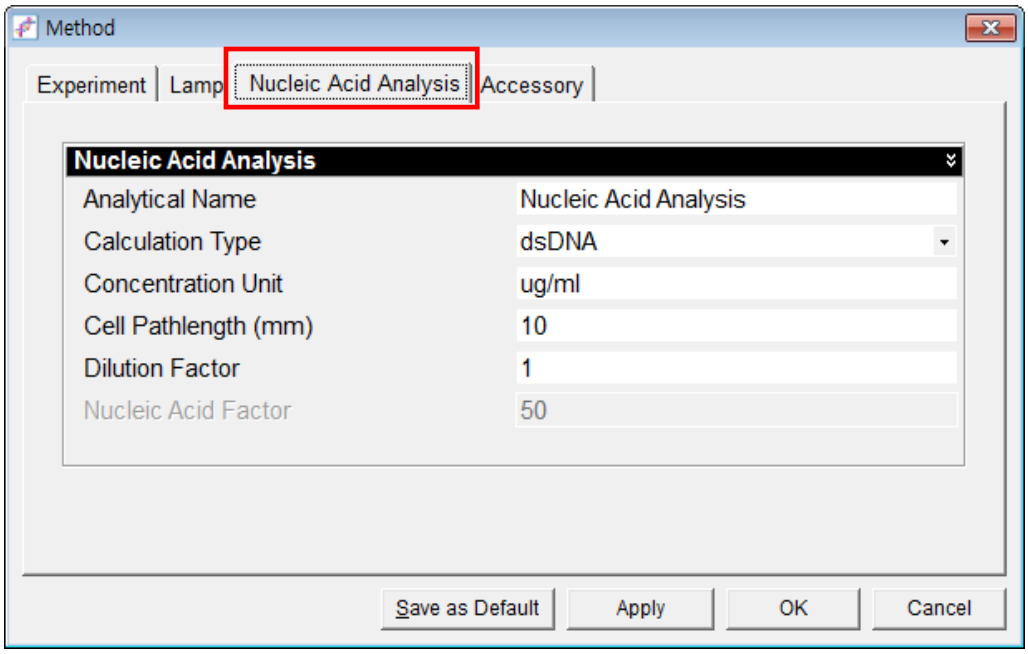
Command	Function
Wavelength (nm)	Default wavelengths are 320, 280, 260, 230 nm.
Y Unit	Displays Y-axis unit Absorbance: (AU) Transmittance: (%T) Reflectance: (%R) Energy
SBW	Select Spectral Band Width (Default: 1.0) 
Measurement No.	Enter the number of repeated sample measurement.
0%T / Blocked Beam Baseline	Check the **0%T/Blocked Beam Baseline** checkbox when measuring samples with high absorbance/low transmittance.

4.2 Lamp



Command	Function
Beam Type	Select Beam Path Type  Single Front: Only uses Sample holder as a single beam type Single Rear: Only uses Reference holder as a single beam type Double Normal: General uses as a double beam type Double Reverse: Reverses the sample and reference position as a double beam type
Lamp	Select Lamp: UV or UV+Vis Default of this mode is UV.

4.3 Nucleic Acid Analysis



Command	Function
Analytical Name	Enter the analytical name.
Calculation Type	Select the calculation type. Calculation Type: dsDNA dsDNA ssDNA RNA Oligo DNA Warburg-Christian Kalb and Bernlohr
Concentration Unit	Enter the concentration unit for the samples.
Cell Pathlength (mm)	Enter the cell (beam) pathlength.
Dilution Factor	Enter the dilution factor.
Nucleic Acid Factor	Will be set automatically according to calculation type. UV Express uses factors 50, 37, 40 and 33 as default settings for dsDNA, ssDNA, RNA and Oligo nucleotides.

4.4 Accessory

For more details of accessory setting refer to each accessory manual.

Calculation Type

The Nucleic Acid application is used for determining the concentration and purity of nucleic acid samples. The reading at 260 nm allows to calculate the concentration.

▶ **dsDNA, ssDNA, RNA, Oligo DNA**

[Nucleic Acid Concentration] ug/ml = A_{260} x extinction coefficient x D.F

- A_{260} is Absorbance at 260 nm.
- Extinction coefficient :

  The generally accepted extinction coefficients for nucleic acids are:

Coefficient	Value
dsDNA	50 ug/ml
ssDNA	37 ug/ml
RNA	40 ug/ml
Oligo DNA	33 ug/ml

- D.F is dilution factor.

▶ **Warburg-Christian**

The Warburg-Christian assay with pre-selected parameters calculates protein and nucleic acid concentrations (in micrograms per ㎖) using the following equations with absorptivities calculated by Warburg and Christian.

[Protein] = (1552 * A_{280}) - (757.3 * A_{260}),
[Nucleic Acid] = (62.9 * A_{260}) – (36.0 * A_{280})

where, the absorbance at 260 nm and 280 nm are corrected for the baseline at 320 nm.

▶ **Kalb and Bernlohr**

The Kalb and Bernlohr assay with pre-selected parameters calculates protein and nucleic acid concentrations (in micrograms per ㎖) using the following equations.

[Protein] = (183.0 * A_{230}) - (75.8 * A_{260}),
[Nucleic Acid] = (49.1 * A_{260}) – (3.48 * A_{230})

where, the absorbance at 260 nm and 230 nm are corrected for the baseline at 320 nm.

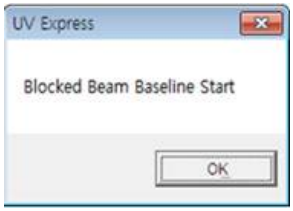
5. After completing parameter setup for Experiment Setup, Lamp and Nucleic Acid Analysis, select **Apply** and then select **OK**.

6. Input the blank solution into Reference and Sample holder both. Close the sample compartment cover and measure Baseline using **Baseline** icon. Absorbance of selected wavelength is changed about 0 AU.

NOTE: *When you check **the 0%T / Blocked Beam Baseline**, Baseline measurement procedure is as follows.*

*a. Click **Baseline** icon.*

*b. When the below message is popped up, click **OK** after checking the empty of both holders (Reference and Sample).*

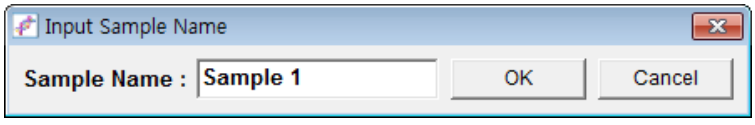


*c. Measure the dark automatically and then, after the below message is shown. Depending on the sample, empty the cell holder or input the blank into reference and sample holder both. Click **OK**.*

NOTE: *You should measure Baseline whenever the wavelength, SBW or Reference sample is changed.*

7. Input the sample into the sample holder and close the sample compartment cover and then, select **Sample** icon.

8. Input sample name and click **OK**.



9. Data will be displayed in the result window.

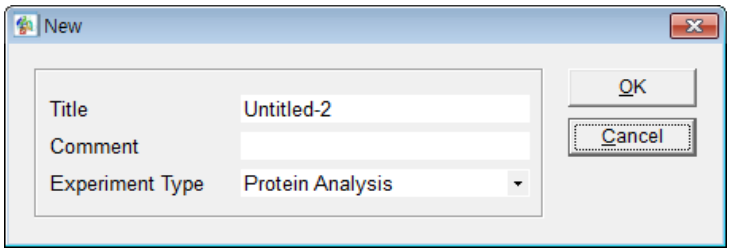
10. Save or print spectrum and results as desired.

X-6. Protein Analysis Mode

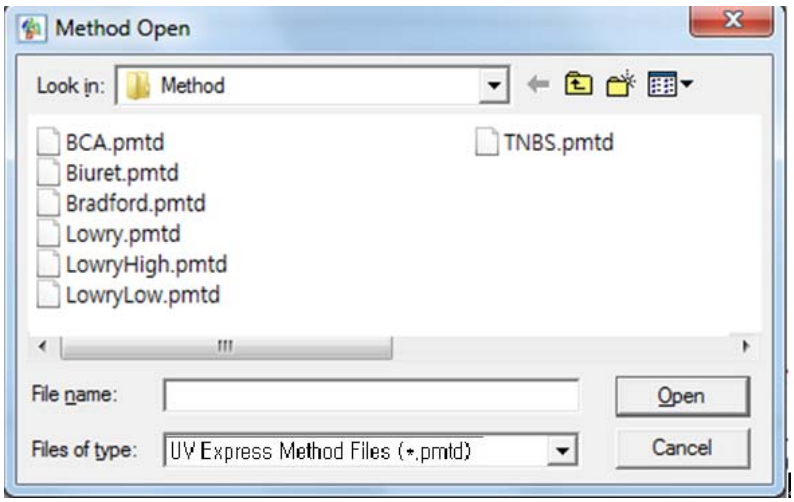
- Use Protein Analysis to quantify the amount of protein in a sample. Select from pre-programmed frequently used methods for quantifying proteins.
- Perform a Protein Analysis Method measurement as follows:
  1. Execute Protein Analysis mode.
  2. Select the protein method.
  3. Set method parameters.
  4. Measure Baseline.
  5. Measure samples.
  6. Save or print results as required.
- Procedure

1. Execute the **Protein Analysis** .

2. The following window will be displayed. Enter Title, Comment and select Experiment Type and then, select **OK**.



3. Select the protein method and select **Open**.



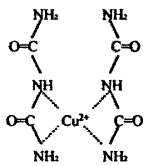
Protein Analysis Method

The study of many biochemical processes depends upon an accurate measurement of the amount of protein in solution. This has led to the development of several protein quantification methods, the most common of which are described below.

Method Name	Reagents	Wavelength [nm]	Concentration Range [µg/ml]	Method File
Biuret	Dilute copper sulfate in strong alkali	540	200–2000	Biuret.pmtd
Lowry (high sensitivity)	Dilute copper sulfate in strong alkali and Folin-Ciocalteu reagent	750	4–200	LowryHigh.pmtd
Lowry (low sensitivity)	Dilute copper sulfate in strong alkali, Folin-Ciocalteu reagent	500	60–400	LowryLow.pmtd
Lowry (modified)	Dilute copper sulfate in strong alkali and Folin-Ciocalteu reagent and dithiothreitol	740	3–200	Lowry.pmtd
Bradford	Goomassie Brilliant Blue G250 in dilute acid	595	2–50	Bradford.pmtd
Bicinchoninic acid (BCA)	Bicinchoninic acid	562	4–400	BCA.pmtd
Trinitrobenzene Sulfonate (TNBS)	Hydrochloric acid and trinitrobenzene reagent	416	0.5–100	TNSB.pmtd

1. Biuret Method

Biuret (NH_2-CO-NH-CO-NH_2) produces a violet chelate compound when reacting with alkali $CuSO_4$.



Using the theory that a compound which has more than two peptide bonds also produces a chelate compound, by the same procedure as in the Biuret case, one can determine the protein concentration. Approximately, **1~10 mg** of protein can be quantified by the Biuret method. Using a microassay one can measure as **0.25 mg~2.0 mg** of protein. A chelate compound is usually stable for 1~2 hours but its chromaticity gradually increases with time. The absorbance of the sample in the test tube is measured at **540 nm**.

2. Lowry Method

The Lowry procedure is one of the most venerable and widely used protein assays, being first described in 1951 [Lowry et al., J. Biol. Chem. 193: 265-275 (1951)]. Under alkaline conditions, copper complexes with protein. When folin phenol reagent (phospho-molybdic-phosphotungstic reagent) is added, the folin-phenol reagent binds to the protein. Bound reagent is slowly reduced and changes color from yellow to blue. The Lowry method is more sensitive than the Biuret method and can measure **10~200 μg** of protein. The absorbance of the sample in the test tube is measured at **750 nm**.

3. Bradford Method

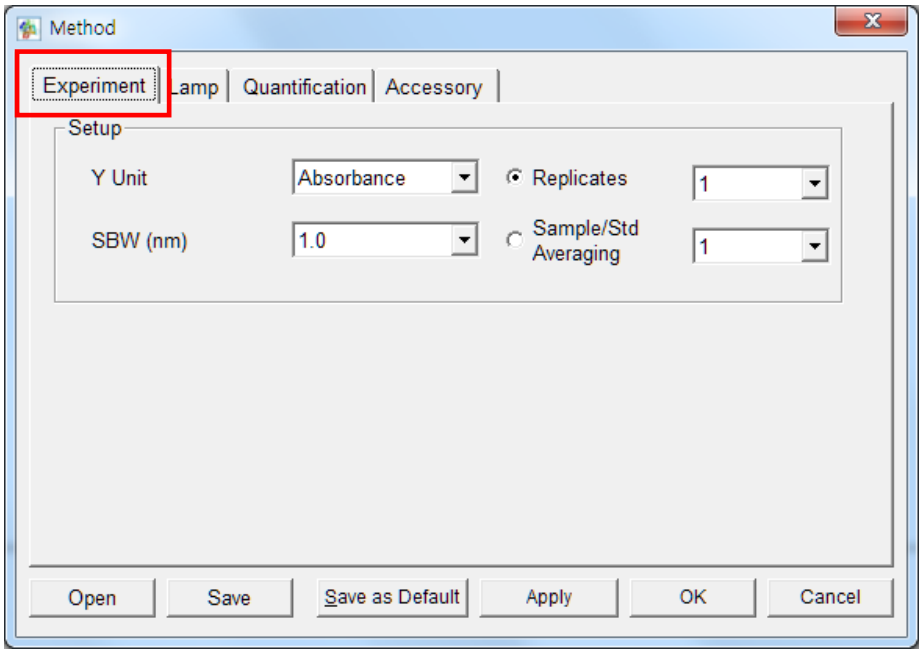
One of the most widely used methods was developed by Bradford; it is based upon a shift in the absorption spectrum of Coomassie Brilliant Blue G-250 when the dye binds to protein in an acidic solution. The method is fast, convenient, and produces an equivalent absorbance change for many different proteins. The absorbance of the resulting solution is measured spectrophotometrically at **595 nm** and compared to a standard curve generated using known quantities of a control protein in the dye solution. In this method, color reaction is completed very quickly (in 2 min.) and it is stable for 1 hour. The Bradford method is more sensitive than the Lowry method and can measure **1~20 μg** of protein using micro assay. The Bradford method is faster and is seldom affected by non-protein components.

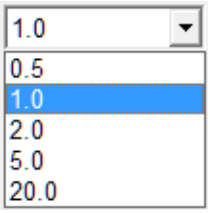
4. BCA Protein Assay

BCA Protein Assay uses a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantification of total protein. This method combines the well-known reduction of Cu^{2+} to Cu^{+} by protein in an alkaline medium (the Biuret reaction) with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{+}) using a unique reagent containing BCA. The purple-colored reaction product in this assay is formed by the chelation of two molecules of BCA with one cuprous ion. This water-soluble complex exhibits a strong absorbance at 562 nm that is nearly linear with increasing protein concentrations over a broad working range (20-2,000 μg/ml). The BCA method is not a true end-point method; i.e., the final color continues to develop. However, following incubation, the rate of continued color development is sufficiently slow to allow large number of samples to be assayed together.

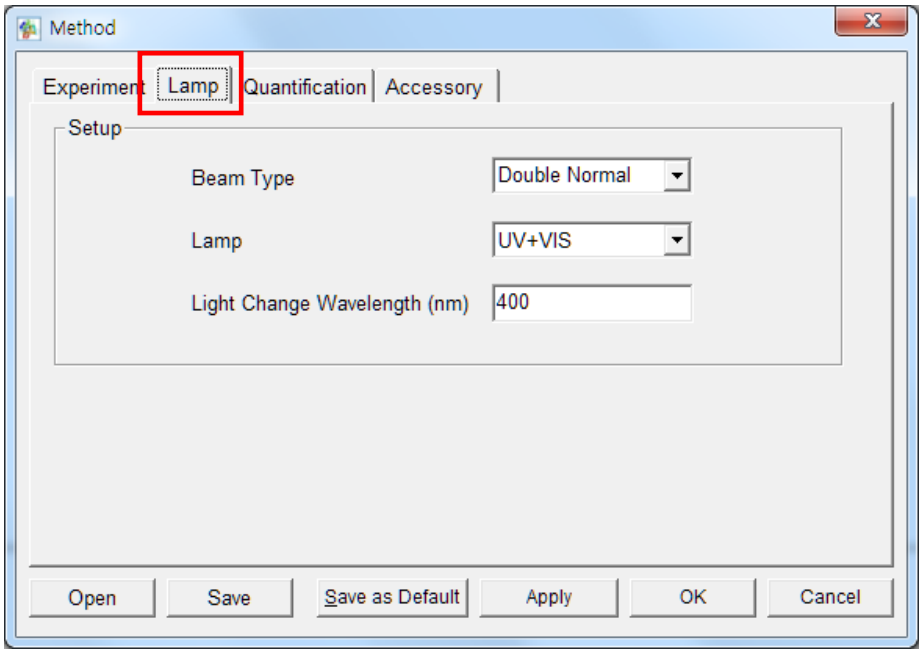
4. Setup the Experiment Setup and Lamp parameters.

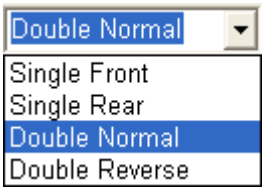
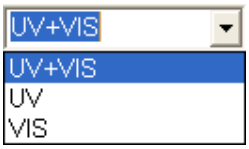
4.1 Experiment Setup



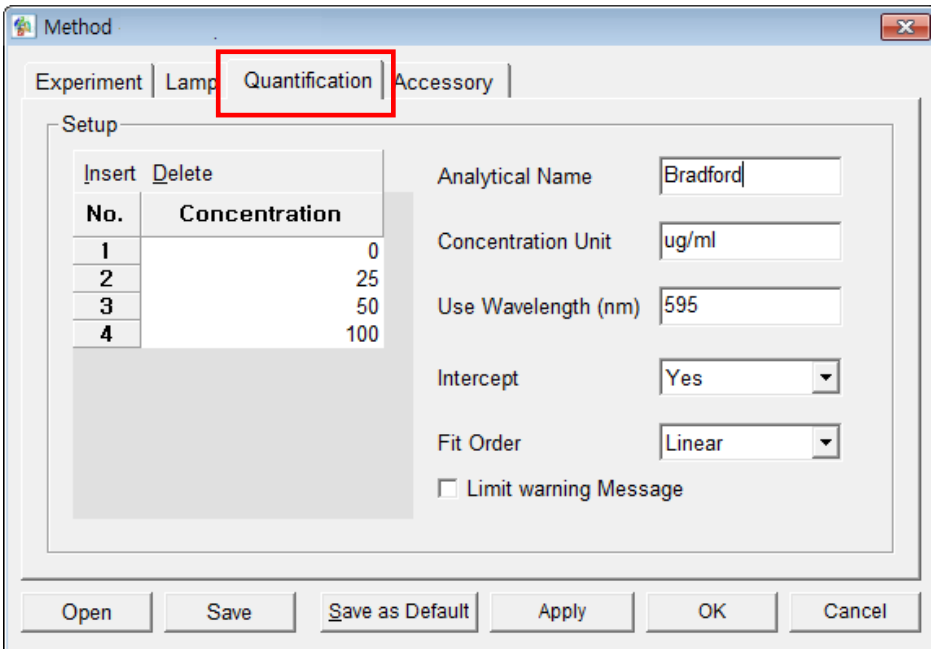
Command	Function
Y Unit	Displays Y-axis unit Absorbance: (AU) Transmittance: (%T) Reflectance: (%R) Energy
SBW	Select Spectral Band Width (Default: 1.0) 
Replicates	Select the number of replicate readings to be taken for each sample (1~5).
Sample/Std Average	Select the number of reading to be taken from different solutions of sample/standards (1~3).

4.2 Lamp



Command	Function
Beam Type	Select Beam Path Type  Single Front: Only uses Sample holder as a single beam type Single Rear: Only uses Reference holder as a single beam type Double Normal: General uses as a double beam type Double Reverse: Reverses the sample and reference position as a double beam type
Lamp	Select Lamp  Light Change Wavelength (nm) will be deactivated if UV or VIS lamp is selected.
Light Change Wavelength (nm)	Sets the changeover wavelength for the deuterium lamp and halogen lamp. Enter a wavelength. (360 ~ 450 nm, default setting: 400 nm)

4.3 Quantification



Command	Function
Setup	Enter each standard concentration using Insert and Delete.
Analytical Name	Enter the file name.
Concentration Unit	Enter the unit of concentration.
Use Wavelength (nm)	Enter the wavelength to test.
Intercept	No: Calibration Curve passes through the origin Yes: Calibration Curve does not pass through the origin
Fit Order	Choose the calibration curve dimension Linear, Quadratic or Cubic.
Limit Warning Message	When the sample measurement value is out of the calibration curve range, the warning message will be shown. It can be selected as an option.

4.4 Accessory

For more details of accessory setting refer to each accessory manual.

5. After completing parameter setup for Experiment Setup, Lamp and Quantification, select **Apply** and then select **OK**.

6. Input the blank solution into Reference and Sample holder both. Close the sample compartment cover and measure Baseline using **Baseline**

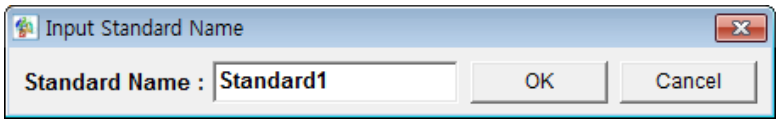
icon. Absorbance of selected wavelength is changed about 0 AU.

NOTE: *You should measure Baseline whenever the wavelength, SBW or Reference sample is changed.*

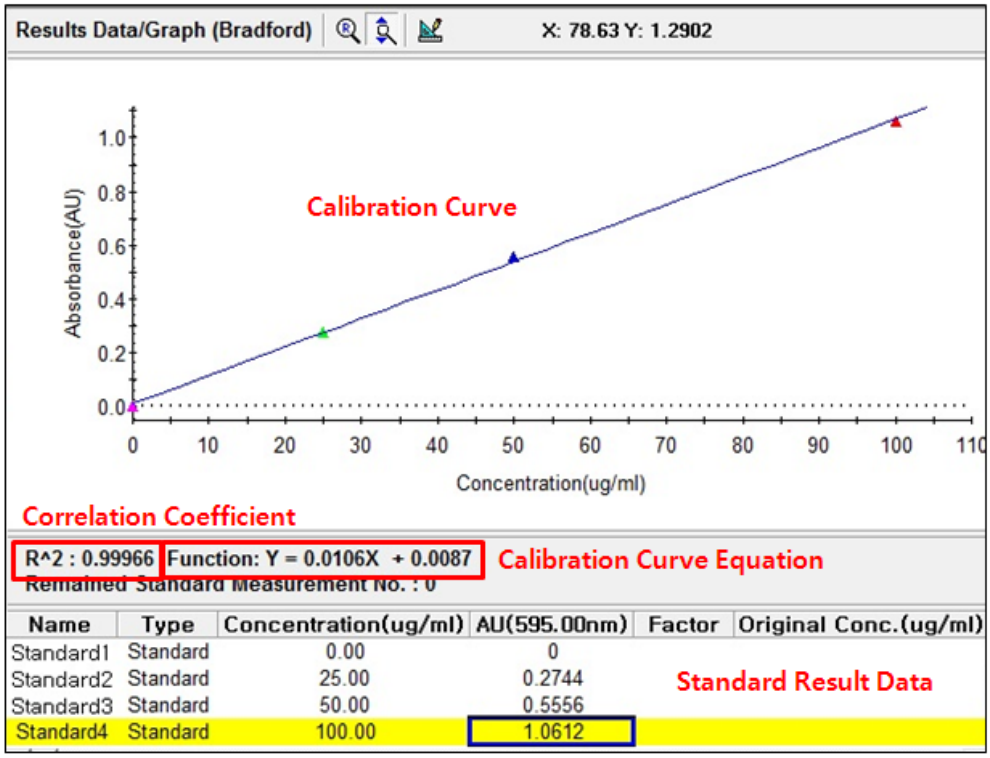
7. Input the standard solution into the Sample holder and then click **Standard**

icon.

8. Input standard name and select **OK**.



9. Measure Standard samples in order and the calibration curve will be created as follows.



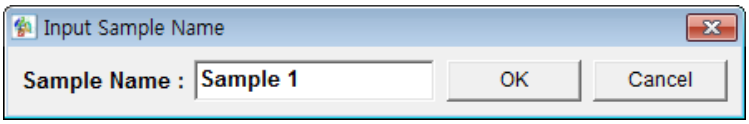
NOTE: *You can save the standard calibration curve and open the curve whenever you need.*

10. Input Blank and Unknown sample into each Reference and Sample holder and then, select **Sample**

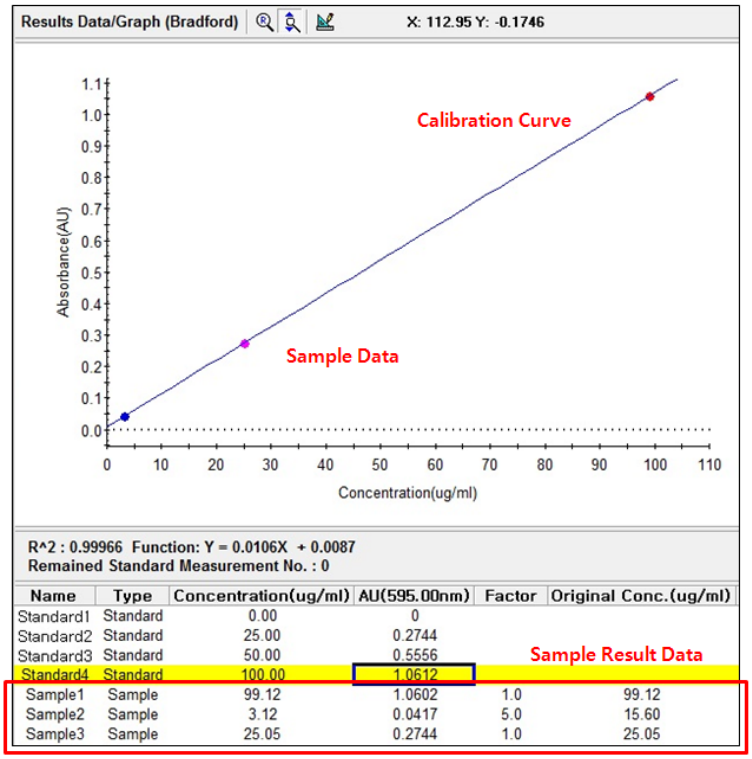
icon.

NOTE: *Click Sample after measuring [Baseline] when you opened the standard curve saved.*

11. Input sample name and select **OK**.



12. Data will be shown as follows.



Name	Type	Concentration(ug/ml)	AU(595.00nm)	Factor	Original Conc.(ug/ml)
Standard1	Standard	0.00	0		
Standard2	Standard	25.00	0.2744		
Standard3	Standard	50.00	0.5556		
Standard4	Standard	100.00	1.0612		
Sample1	Sample	99.12	1.0602	1.0	99.12
Sample2	Sample	3.12	0.0417	5.0	15.60
Sample3	Sample	25.05	0.2744	1.0	25.05

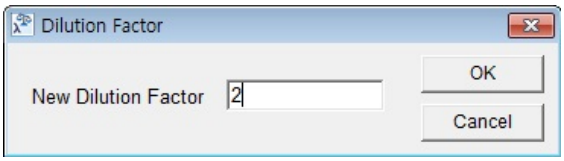
a. Name: Shows the entered Standard or Sample name.

b. Type: Displays the Standard or Sample.

c. Concentration: Display the calculated concentration.

d. AU: Displays the measured Y-axis value.

e. Dilution factor: The dilution factor is set 1.0 as a default and it can be edited after the sample measurement. Double-click on the Dilution Factor to be changed in each sample measurement result and enter a new Dilution Factor and click **OK**.

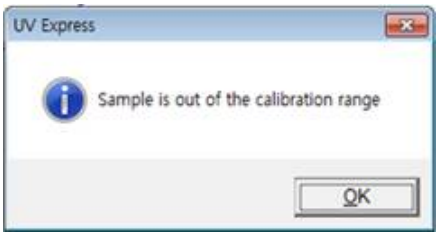


f. Original Conc.: Shows the original concentration of sample.

(Original Concentration = Concentration x Dilution Factor)

g. SD, %RSD: Shows the statistics of the repeated measures data.

NOTE: *In case of checking on Limit warning Message in the Quantification tab of the Method window, the following message is shown as the sample measurement values is out of the calibration curve range.*



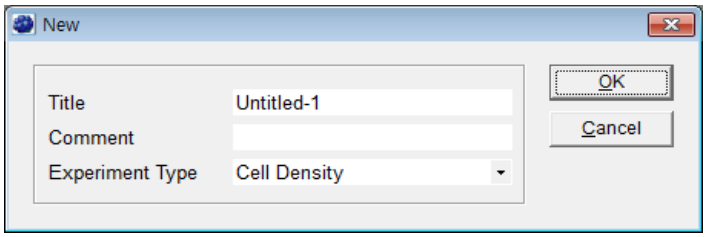
13. Save or print spectrum and results as desired.

X-7. Cell Density Mode

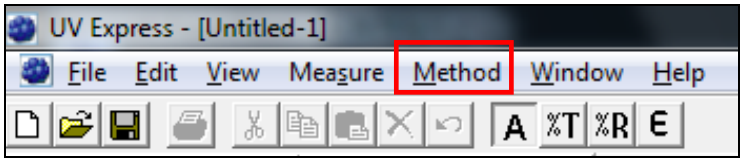
- Use Cell Density to determinate the absorbance at 600 nm.
- Perform a Cell Density Method measurement as follows:
  1. Execute Cell Density mode.
  2. Set method parameters.
  3. Measure Baseline.
  4. Measure samples.
  5. Save or print results as required.
- Procedure

1. Execute the **Cell Density** .

2. The following window will be displayed. Enter Title, Comment and select Experiment Type and then, select **OK**.

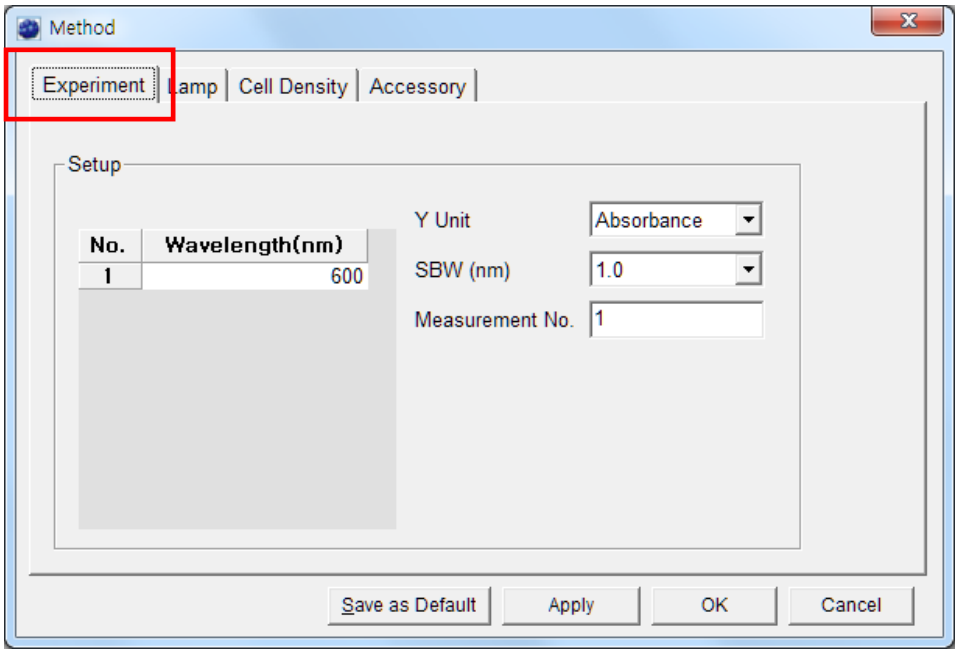


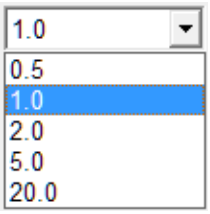
3. Select **Method**.



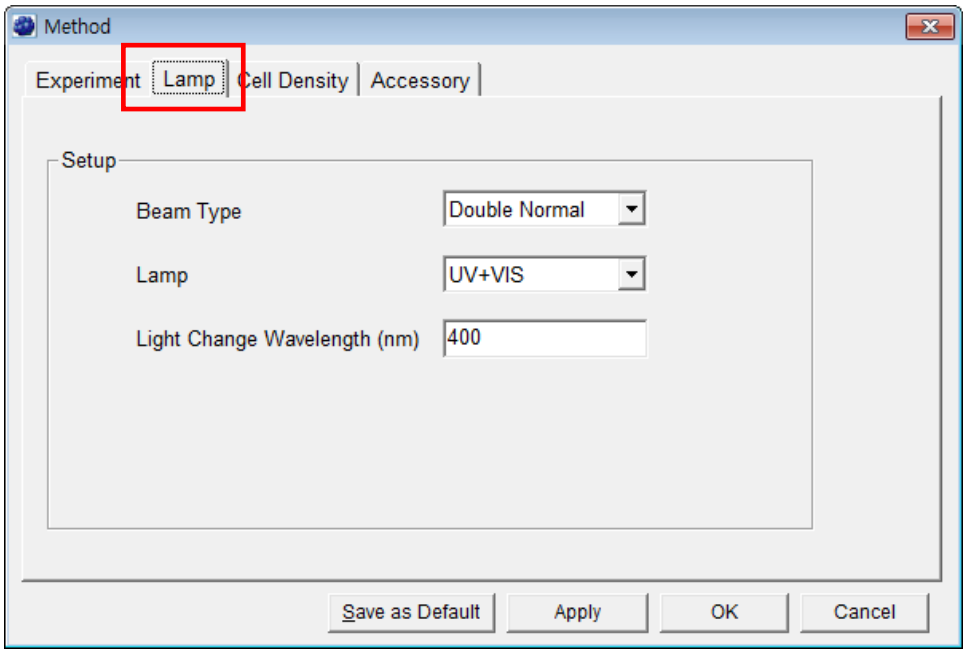
4. Setup the Experiment Setup and Lamp parameters.

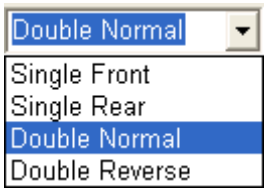
4.1 Experiment



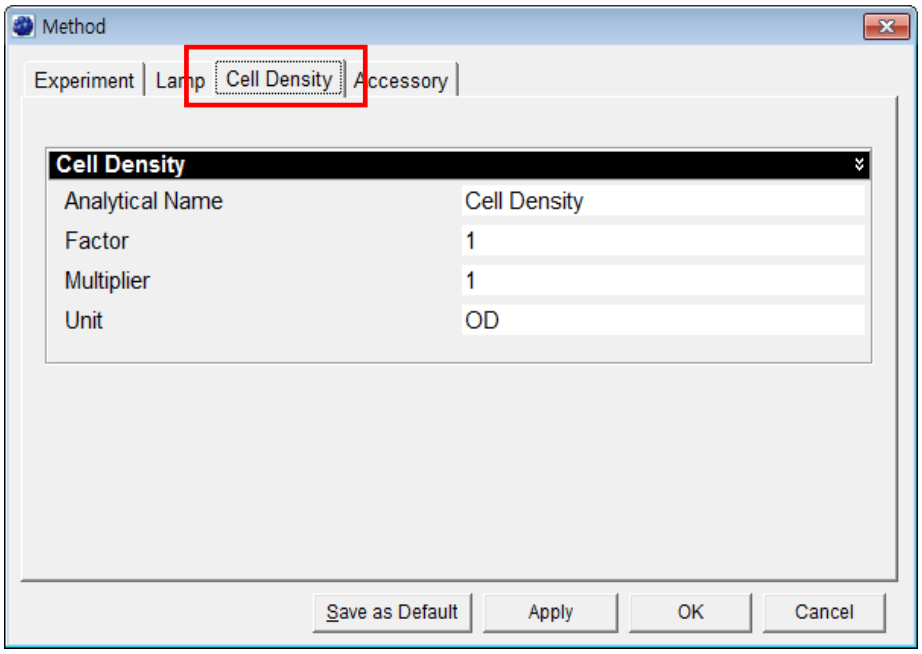
Command	Function
Wavelength (nm)	Default wavelengths are 600 nm.
Y Unit	Displays Y-axis unit. Absorbance: (AU) Transmittance: (%T) Reflectance: (%R) Energy
SBW	Select Spectral Band Width (Default: 1.0). 
Measurement No.	Enter the number of repeated sample measurement.

4.2 Lamp



Command	Function
Beam Type	Select Beam Path Type  Single Front: Only uses Sample holder as a single beam type Single Rear: Only uses Reference holder as a single beam type Double Normal: General uses as a double beam type Double Reverse: Reverses the sample and reference position as a double beam type
Lamp	Select Lamp: UV + VIS, UV or VIS Light Change Wavelength (nm) will be deactivated if UV or VIS lamp is selected.
Light Change Wavelength (nm)	Sets the changeover wavelength for the deuterium lamp and halogen lamp. Enter a wavelength. (360~450 nm, default setting: 400 nm)

4.3 Cell Density



Command	Function
Analytical Name	Enter the analytical name.
Factor	Set a desired factor value. [used when entering cell/ml in Units]
Multiplier	Set a desired Multiplier value. [used when entering cells/ml in Units] When entering OD in Units, set the Factor and Multiplier as 1. Factor and Multiplier define the conversion of the measured OD to the number of cells per milliliter (e.g.: Factor 5, Multiplier: 100,000,000) 1 OD 600 = 5 x 10^8 cells/ml
Units	Enter OD or cells/ml.

4.4 Accessory

For more details of accessory setting refer to each accessory manual.

5. After completing parameter setup for Experiment, Lamp and Cell Density, click **Apply** and then click **OK**.

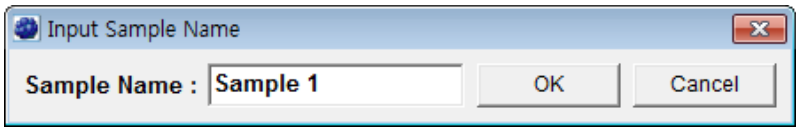
6. Input the blank solution into Reference and Sample holder both. Close the sample compartment cover and measure Baseline using **Baseline**

icon. Absorbance of selected wavelength is changed about 0 AU.

NOTE: *You should measure Baseline whenever the wavelength, SBW or Reference sample is changed.*

7. Input the sample into the sample holder and close the sample compartment cover and then, select **Sample** icon.

8. Input sample name and click **OK**.



9. Data will be displayed in the result window.

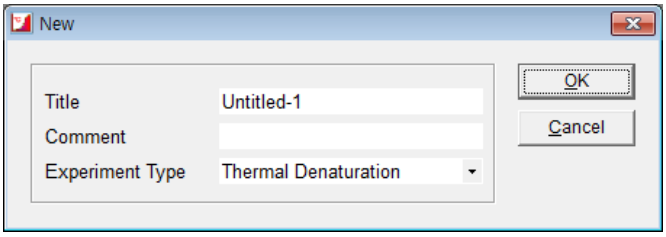
10. Save or print spectrum and results as desired.

X-8. Thermal Denaturation Mode

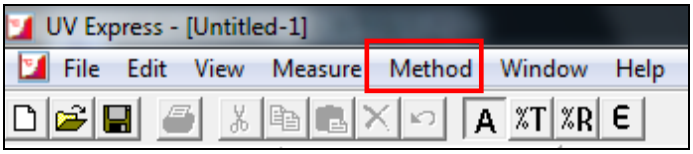
- Use Thermal Denaturation mode to collect the temperature based data and perform a classical DNA melting experiment.
- Perform a Thermal Denaturation Method measurement as follows:
  1. Execute Thermal Denaturation mode.
  2. Set method parameters.
  3. Measure Baseline.
  4. Measure samples.
  5. Save or print results as required.
- Procedure

1. Execute the **Thermal Denaturation** **.**

2. The following window will be displayed. Enter **Title, Comment** and select **Experiment Type** and then, select **OK**.

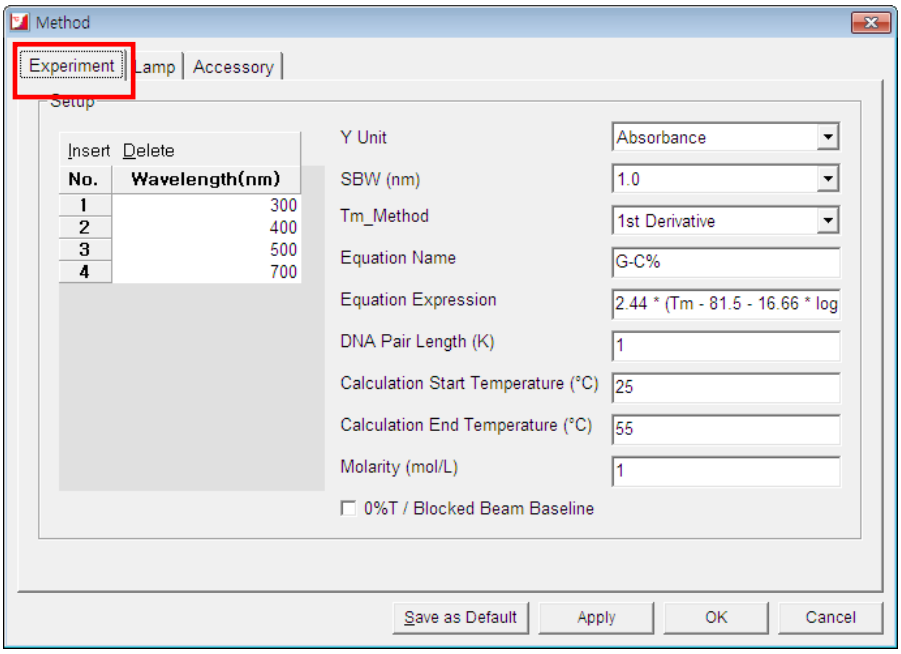


3. Select **Method**.



4. Setup the Experiment, Lamp and Accessory parameters.

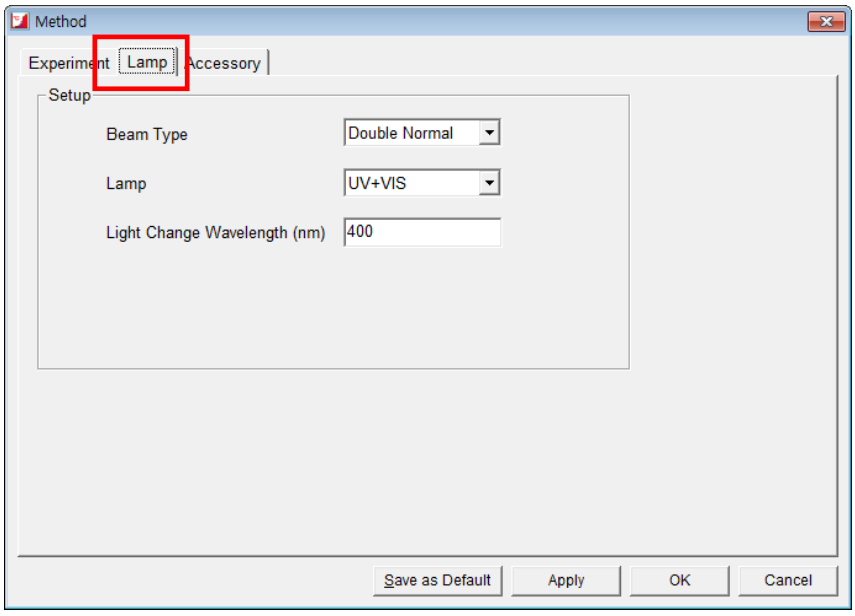
4.1 Experiment

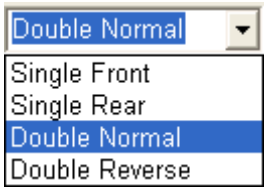
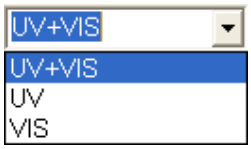


Command	Function
Wavelength (nm)	Enter the wavelength using **Insert** and **Delete**. If the 6-Position Peltier Controlled Cell Changer is used, the number of wavelength should be the same as the number of Using Cell and the Wavelength Range number will coincide with the cell position order in the Using Cells in the Multi-Cell Holder Setup window.
Y Unit	Displays Y-axis unit: Absorbance (AU), Transmittance: (%T) Reflectance: (%R) or Energy
SBW	Select Spectral Band Width: 0.5, 1.0, 2.0, 5.0, and 20.0 nm. Default is 1.0 nm
Tm_Method	Select a method for determining Tm (DNA melting temperature). Options include: 1st derivative and Average.
Equation Name	Enter the name of the formula. %G-C is entered as default, of which equation is formulated in **Equation Expression**.

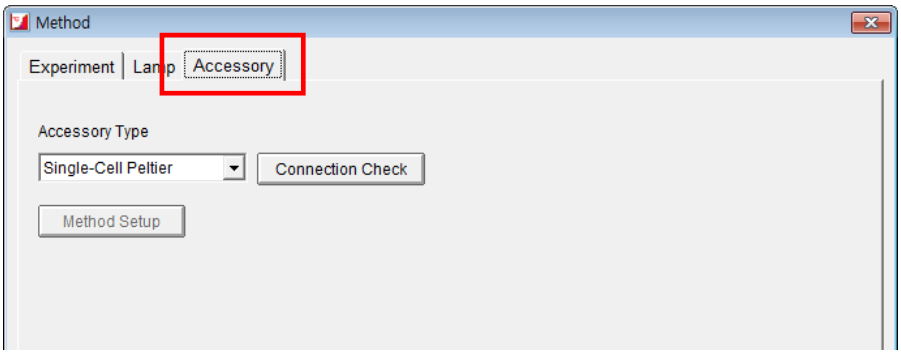
Equation Expression	The default equation for the calculation of %G-C (Guanine-Cytosine) base pairs is: **%G-C=2.44 * (Tm – 81.5 - 16.66 * log(M) + 500/K)** Where M is the molarities of salt in mol/L, K is the DNA base pair length. %G-C will be calculated with estimated T_m and input values, Molarities (M) and length of DNA (K). If a DNA base pair length (K) is entered as '0', then the equation becomes: **%G-C= 2.44 * (Tm - 81.5- 16.66 * log(M))**
DNA Pair Length (K)	Enter the DNA pair length. If a DNA pair length is above 5000, enter as "0".
Calculation Start Temperature (℃)	Enter the start temperature for calculating the Tm value using the selected method.
Calculation End Temperature (℃)	Enter the end temperature for calculating the Tm value using the selected method.
Molarity (mol/L)	Enter the salt molarities of the solution [mol/L]. This value will be used calculating %G-C.
0%T / Blocked Beam Baseline	Check the **0%T/Blocked Beam Baseline** checkbox when measuring samples with high absorbance/low transmittance.

4.2 Lamp



Command	Function
Beam Type	Select Beam Path Type  Single Front: Only uses Sample holder as a single beam type Single Rear: Only uses Reference holder as a single beam type Double Normal: General uses as a double beam type Double Reverse: Reverses the sample and reference position as a double beam type
Lamp	Select Lamp  Light Change Wavelength (nm) will be deactivated if UV or VIS lamp is selected.
Light Change Wavelength (nm)	Sets the changeover wavelength for the deuterium lamp and halogen lamp. Enter a wavelength. (360~450 nm, default setting: 400 nm)

4.3 Accessory



In the Thermal Denaturation mode, select Single Cell Peliter or Multi-Cell Peltier accessory.

For more details of the Peltier Accessory temperature setting, refer to the 6-Position Peltier Controlled Cell Changer (09931286 or Single Cell Peltier Holder manual.

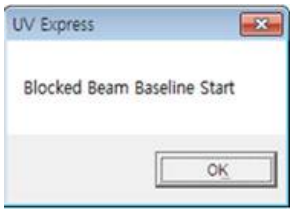
5. After completing parameter setup for Experiment, Lamp and Accessory, click **Apply** and then select **OK**.

6. Input the blank into Reference and Sample holder both. Close the sample compartment cover and measure Baseline using **Baseline** icon. Baseline will be measured when it reaches the set start temperature.

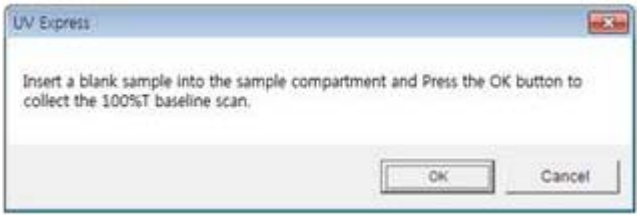
NOTE: *When you check **the 0%T / Blocked Beam Baseline**, Baseline measurement procedure is as follows.*

*a. Click **Baseline** icon.*

*b. When the below message is popped up, click **OK** after checking the empty of both holders (Reference and Sample).*



*c. Measure the dark automatically and then, after the below message is shown. Depending on the sample, empty the cell holder or input the blank into reference and sample holder both. Click **OK**.*



NOTE: *You should measure Baseline whenever the wavelength, SBW or Reference sample is changed.*

7. Remove blank solution from sample cell holder and Input the sample solution into the sample holder. Close the sample compartment cover and then, select **Sample** icon. The measurement will be started after the temperature reached the target temperature.

8. After the measurement is finished, the results are displayed in the result window. Save or print results as required.

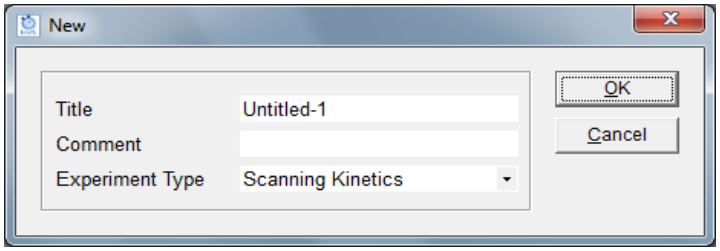
X-9. Scanning Kinetics Mode

- Use Scanning Kinetics to test reaction rate among the specified wavelength range. Scanning Kinetics mode measures the changes to a sample's absorbance, transmittance and reflectance over time at a specified wavelength range.
- Perform a Scanning Kinetics Method measurement as follows:
  1. Select measurement mode.
  2. Set method parameters.
  3. Measure Baseline.
  4. Measure samples.
  5. Save or print results as required.

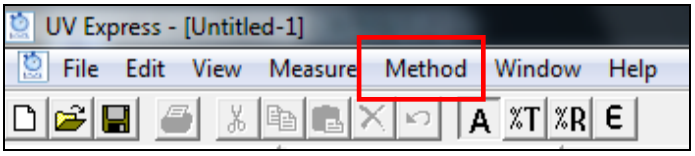
- Procedure

1. Execute the **Scanning Kinetics** .

2. The following window will be displayed. **Enter Title, Comment** and select **Experiment Type** and then, select **OK**.

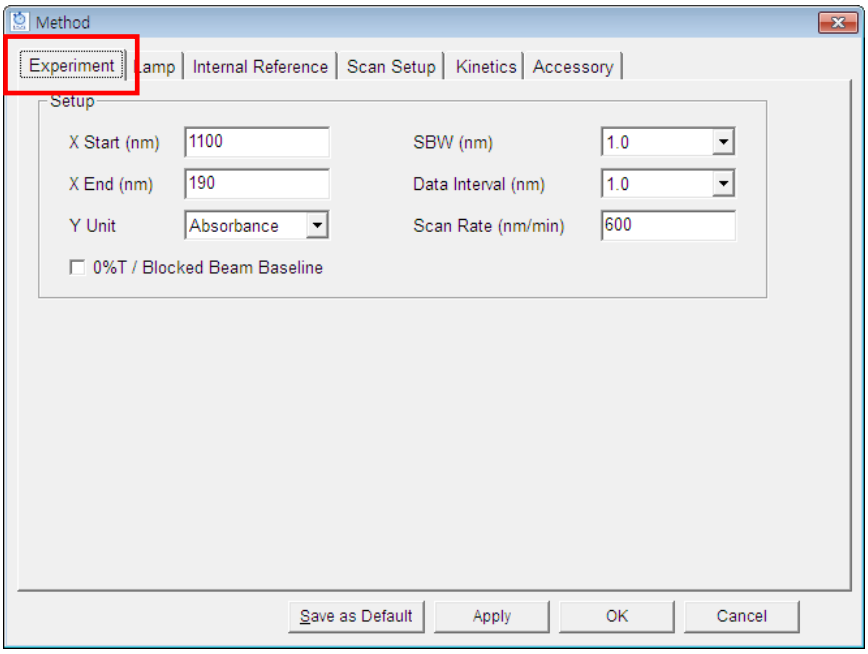


3. Select **Method**.



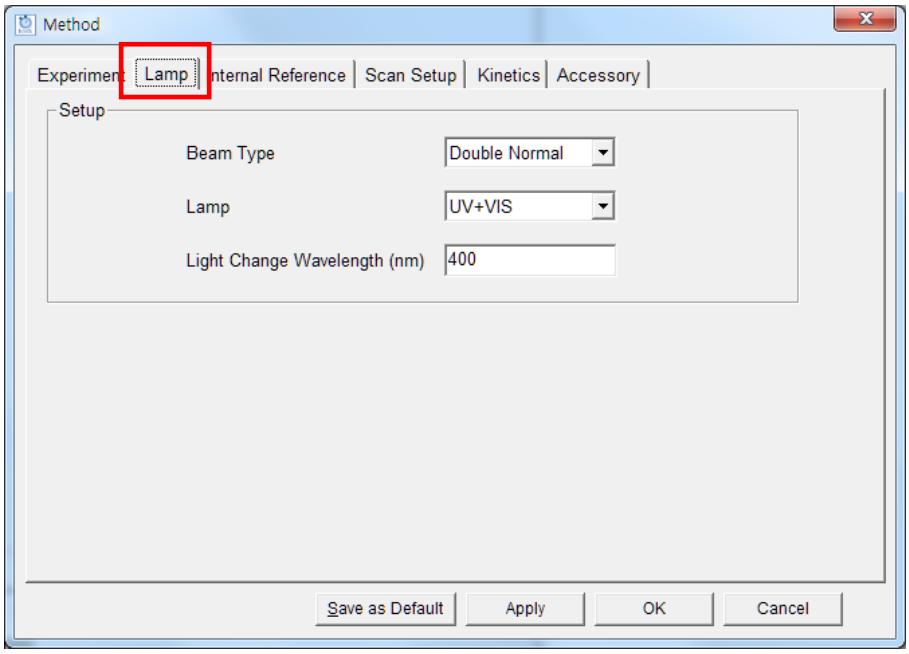
4. Setup the experiment, Lamp and Kinetics parameters.

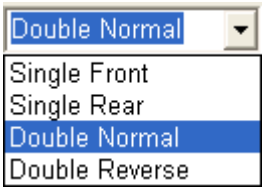
4.1 Experiment



Command	Function
X Start Value	Input the start measurement wavelength (190 to 1100 nm)
X End Value	Inputs the end measurement wavelength
Y Unit	Select Y unit. Absorbance (AU), Transmittance (%T), Reflectance (%R) or Energy
SBW	Select Spectral Band Width. 1.0 0.5 1.0 2.0 5.0 20.0 Default is 1.0 nm.
Data Interval (nm)	Select Data Interval. (0.05, 0.1, 0.5, 1.0 or 2.0 nm) Default is 1.0 nm
Scan Rate (nm/min)	Shows the scan speed per time
0%T / Blocked Beam Baseline	Check the **0%T/Blocked Beam Baseline** checkbox when measuring samples with high absorbance/low transmittance.

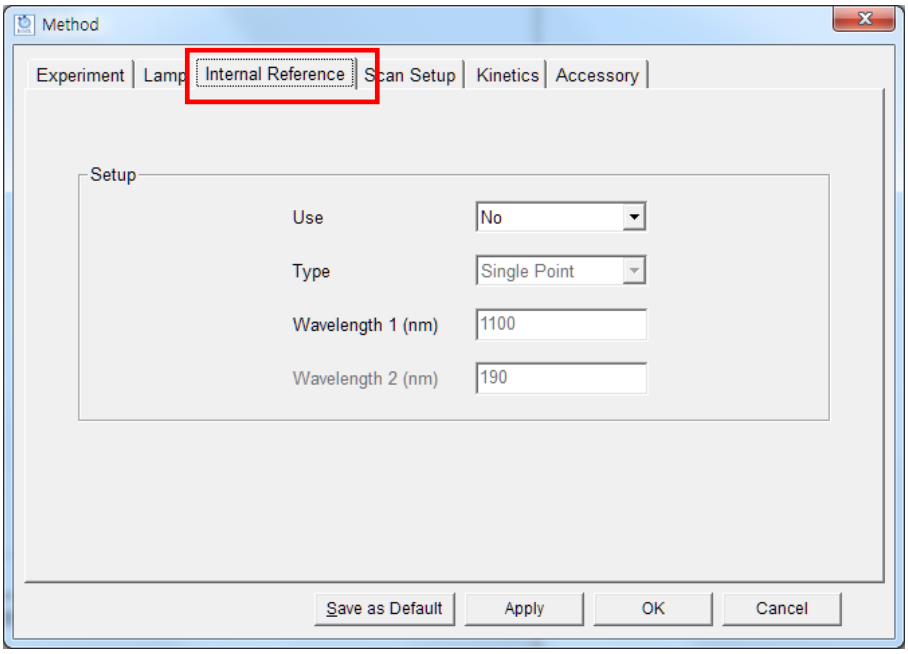
4.2 Lamp



Command	Function
Beam Type	Select Beam Path Type  Single Front: Only uses Sample holder as a single beam type Single Rear: Only uses Reference holder as a single beam type Double Normal: General uses as a double beam type Double Reverse: Reverses the sample and reference position as a double beam type
Lamp	Select Lamp: UV+Vis, UV or VIS Light Change Wavelength (nm) will be deactivated if UV or VIS lamp is selected.
Light Change Wavelength (nm)	Sets the changeover wavelength for the deuterium lamp and halogen lamp. Enter a wavelength. (360~450 nm, default setting: 400 nm)

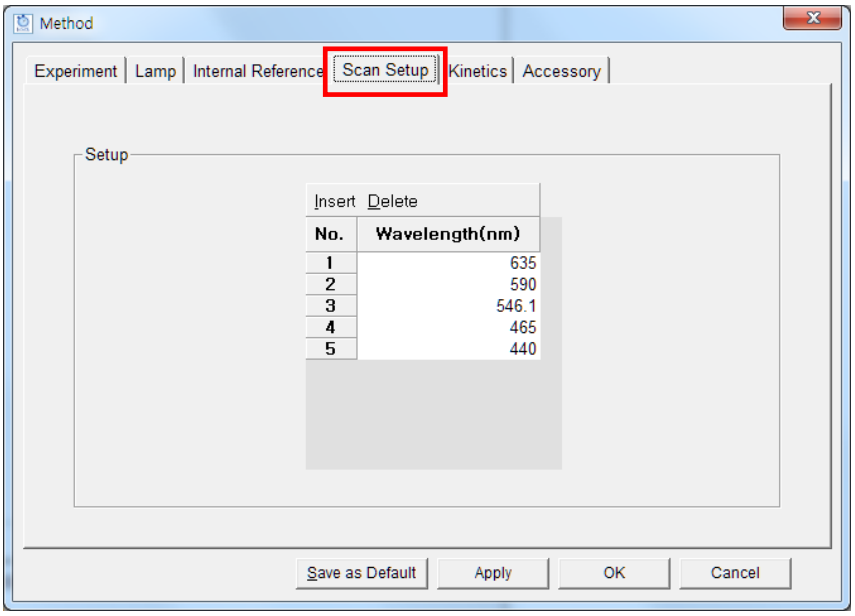
4.3 Internal Reference

Internal Reference is a technique that can be used to improve the precision of results by minimizing the effects of any changes that cause a linear baseline shift, for example a drift in lamp intensity. For more details, please refer to 4.3 Internal Reference in X-1-1. Scan Setup.

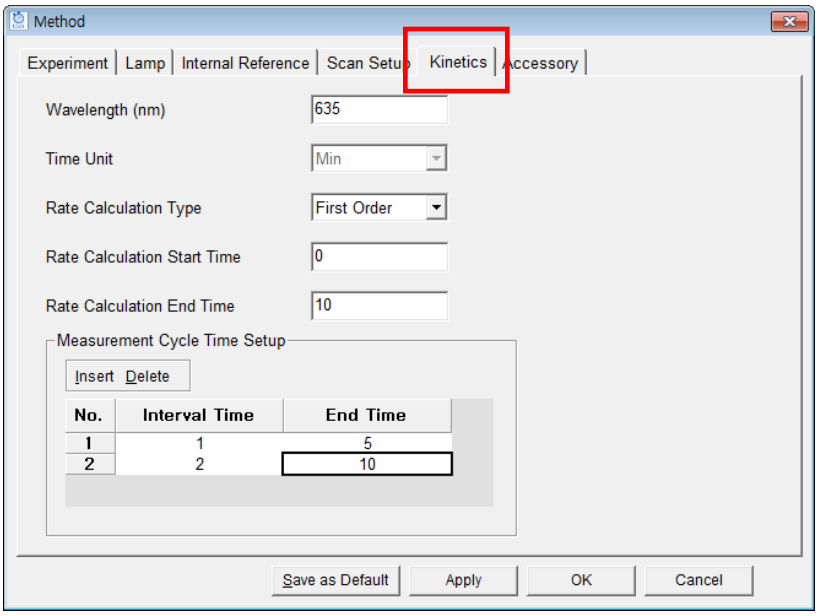


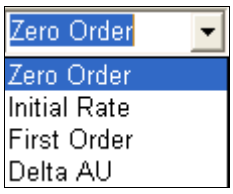
4.4 Scan Setup

Enter the wavelength using **Insert** and **Delete**.



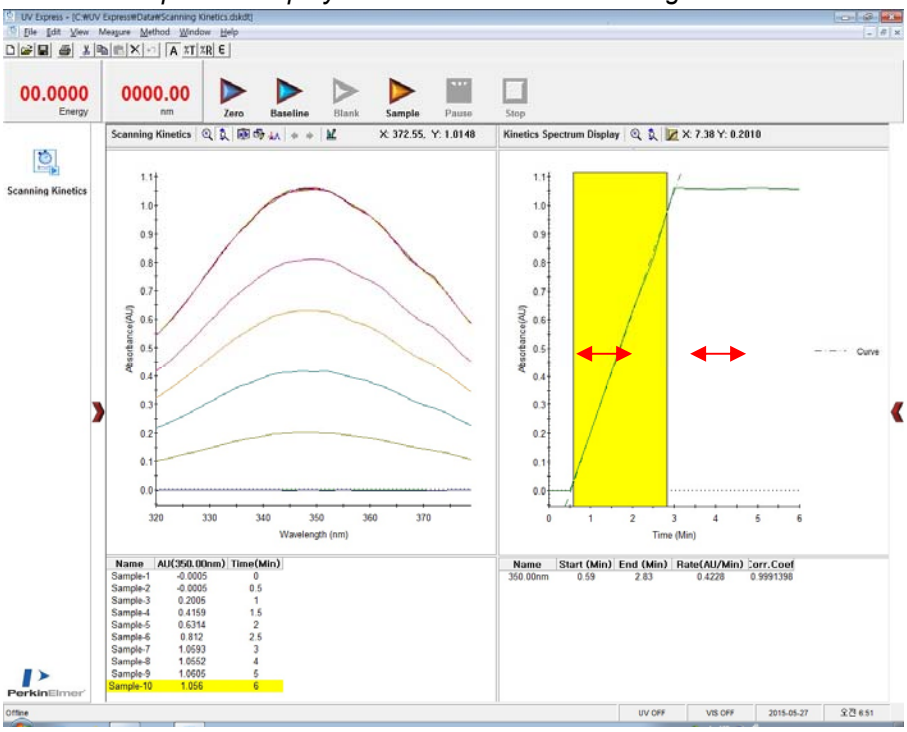
4.5 Kinetics



Command	Function
Wavelength (nm)	Enter the desired wavelength for the Rate calculation
Time Unit	Choose a time unit either sec or min
Rate Calculation Type	Select Calculation Type  ▶ **Zero Order** The zero order calculation uses a linear fit to calculate the rate, k, b linear regression using the equation: **$A_t = A_0 + kt$** **A_t** is the absorbance at time **t** **A_0** is the absorbance at the start of the calculation time range **k** is the zero order rate constant [Units: 1/ AU·s] ▶ **Initial Rate** The initial rate calculation uses a quadratic fit to calculate the rate, k, by linear regression using the equation:

	$A_t = A_0 + kt + t^2$ Where A_t is the absorbance at time **t** A_0 is the initial absorbance **k** is the initial rate [Units: AU/s] **First order** The first order calculation uses an exponential fit to calculate the rate, k, using: $A_t = A_\infty + (A_o - A_\infty) e^{-kt}$ This rate law can be rearranged to $\ln(A_t - A_\infty) = \ln(A_o - A_\infty) - kt$ Where A_t is the absorbance at time **t** A_0 is the initial absorbance **k** is the first order rate constant [Unit : 1/s] ▶ **Delta AU** Delta AU is the difference between the absorbance at the start of the calculation time range and the absorbance at the end. The calculation is very simple: **Delta AU = At - A0** Where **At** is the absorbance at time **t** A_0 is the initial absorbance **Delta AU** [Units: AU]
Rate Calculation Start Time	Enter the start time to calculate the reaction rate
Rate Calculation End Time	Enter the finish time to calculate the reaction rate
Measurement Cycle Time Setup	The whole measurement time can be split a number of time phases. Insert or delete the phase and set the time properties.
Interval Time	Enter the interval time between measurements
End Time	Input the end time for Kinetic Rate calculation

NOTE: *User can change the rate calculation range roughly by dragging the mouse. Select Cursor* *icon in Spectral Display Function Tool to set the range.*



4.6 Accessory

For more details of accessory setting refer to each accessory manual.

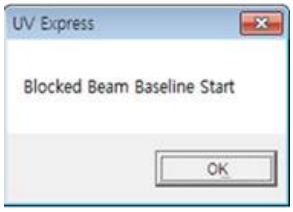
5. After completing parameter setup for Experiment, Lamp, Internal Reference, Scan Setup and Kinetics, select **Apply** and then select **OK**.

6. Input the blank solution into Reference and Sample holder both. Close the sample compartment cover and measure Baseline using **Baseline** icon. Absorbance of selected wavelength is changed about 0 AU.

NOTE: *When you check **the 0%T / Blocked Beam Baseline**, Baseline measurement procedure is as follows.*

*a. Click **Baseline** icon.*

*b. When the below message is popped up, click **OK** after checking the empty of both holders (Reference and Sample).*

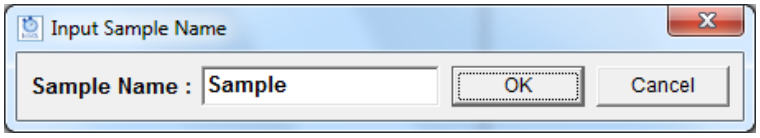


c. *Measure the dark automatically and then, after the below message is shown. Depending on the sample, empty the cell holder or input the blank into reference and sample holder both. Click* ***OK****.*

NOTE: *You should measure Baseline whenever the wavelength, SBW or Reference sample is changed*

7. Remove blank solution from sample cell holder and Input the sample solution into the sample holder. Close the sample compartment cover and then, select **Sample** icon. The measurement will be started after the temperature reached the target temperature.

8. Input sample name and select **OK**.



9. If you want to stop for a while on the way of measurement, select **Pause** icon. Resume by selecting **Restart** icon.

10. After the experiment is completed, confirm the result data.

11. Save or print spectrum and results as desired.

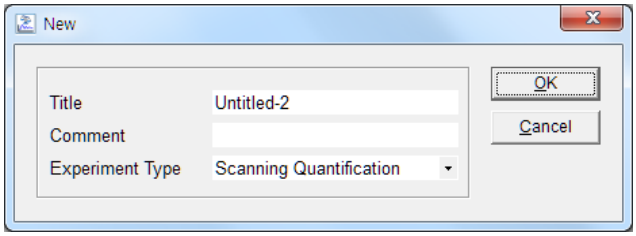
X-10. Scanning Quantification Mode

- Scanning Quantification mode allows quantifying the unknown samples' concentration based on standards calibration curve at the specified wavelength among the one of the measured spectral range.
- Use Scanning Quantification mode to quantify a sample at one of the scanned wavelength using a reference standard.
- Perform a Scanning Quantification Method measurement as follows:
  1. Select measurement mode.
  2. Set method parameters.
  3. Measure Baseline.
  4. Scan with standards. Create the calibration curve using a set of standards at the specified wavelength.
  5. Scan samples.
  6. Save or print results as required.

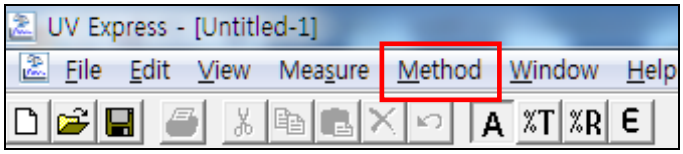
- Procedure

1. Execute the **Scanning Quantification** .

2. The following window will be displayed. Enter Title, Comment and select Experiment Type and then, click **OK**.

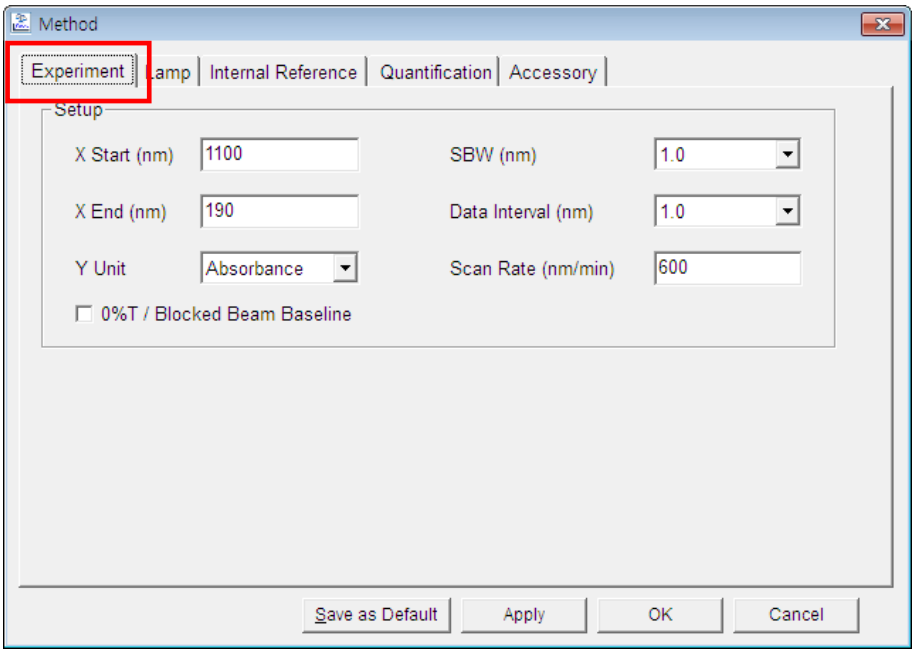


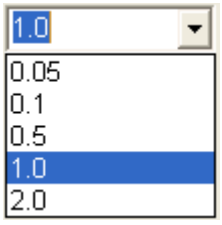
3. Select **Method**.



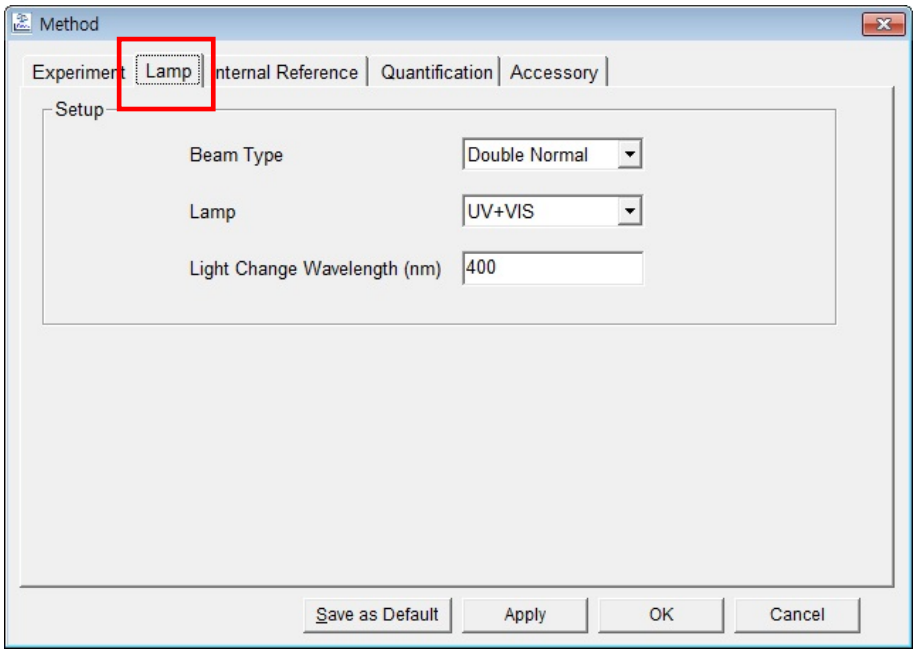
4. Setup the experiment, Lamp and Quantification parameters.

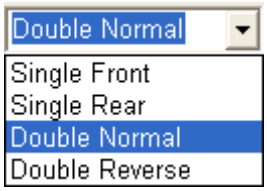
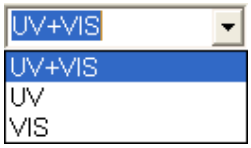
4.1 Experiment



Command	Function
X Start (nm)	Input the start measurement wavelength (190 to 1100 nm)
X End (nm)	Inputs the end measurement wavelength
Y Unit	Displays Y-axis unit: Absorbance (AU), Transmittance (%T) Reflectance (%R) or Energy
SBW	Select Spectral Band Width: 0.5, 1.0, 2.0, 5.0 or 20 nm. Default is 1.0 nm.
Data Interval (nm)	Select Data Interval (Default: 1.0 nm) 
Scan Rate (nm/min)	Shows the scan speed per time.
0%T / Blocked Beam Baseline	Check the **0%T/Blocked Beam Baseline** checkbox when measuring samples with high absorbance/low transmittance.

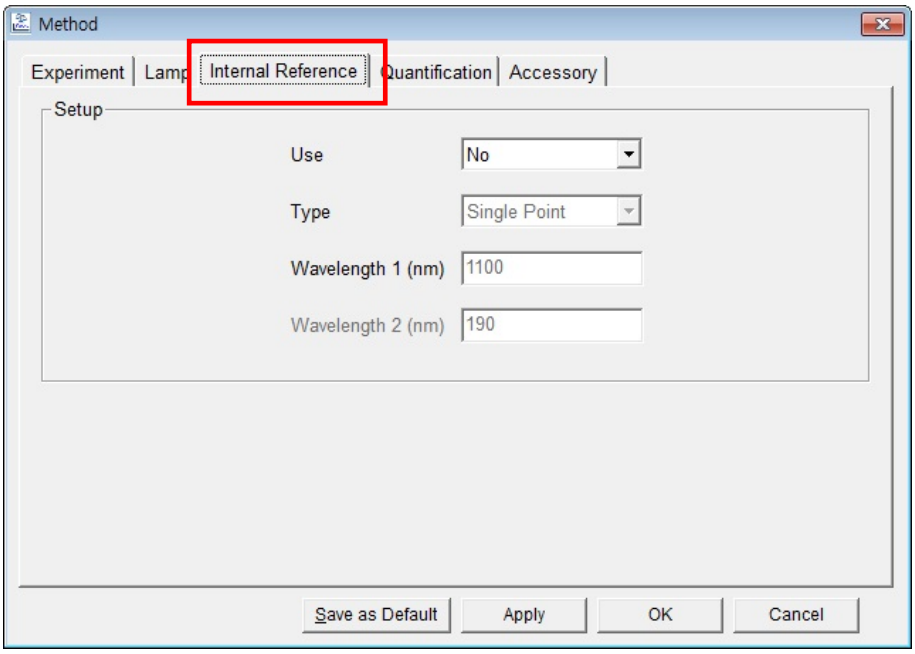
4.2 Lamp



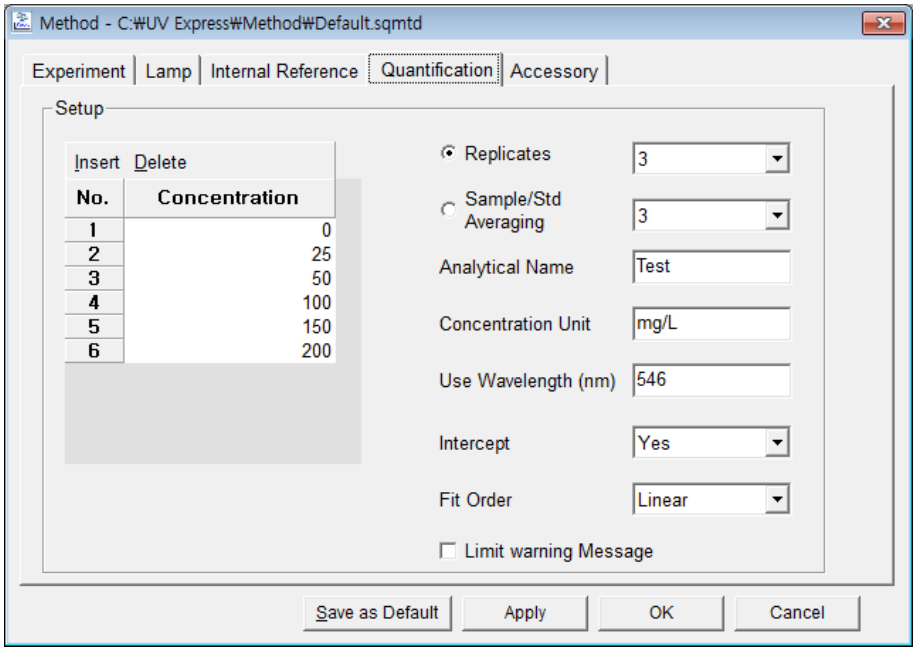
Command	Function
Beam Type	Select Beam Path Type.  Single Front: Only uses Sample holder as a single beam type Single Rear: Only uses Reference holder as a single beam type Double Normal: General uses as a double beam type Double Reverse: Reverses the sample and reference position as a double beam type
Lamp	Select Lamp.  Light Change Wavelength(nm) will be deactivated if UV or VIS lamp is selected
Light Change Wavelength (nm)	Sets the changeover wavelength for the deuterium lamp and halogen lamp. Enter a wavelength (360~450 nm, default setting: 400 nm)

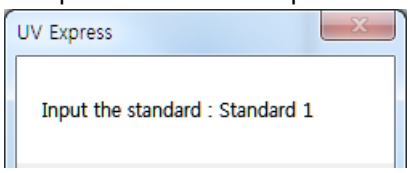
4.3 Internal Reference

Internal Reference is a technique that can be used to improve the precision of results by minimizing the effects of any changes that cause a linear baseline shift, for example a drift in lamp intensity. For more details, please refer to 4.3 Internal Reference in X-1-1. Scan Setup.



4.4 Quantification



Command	Function
Setup	Enter each standard concentration using Insert and Delete.
Replicates	Select the number of replicate readings to be taken for each sample (1~5). The measurement is automatically repeated as set number.
Sample/Std Averaging	Select the number of reading to be taken from different solutions of sample/standards (1~3). Sample/Std has to be replaced between each count. 
Analytical Name	Enter the file name.
Concentration Unit	Enter the unit of concentration.
Use Wavelength (nm)	Enter the wavelength to test.
Intercept	No: Calibration Curve passes through the origin Yes: Calibration Curve does not pass through the origin
Fit Order	Choose the calibration curve dimension: Linear, Quadratic or Cubic
Limit warning Message	When the measured sample value is out of calibration curve range, the below warning message will be shown. This message can be checked as optional.

4.5 Accessory

For more details of accessory setting refer to each accessory manual.

5. After completing parameter setup for Experiment, Lamp, Internal Reference and Quantification, select **Apply** and then select **OK**.

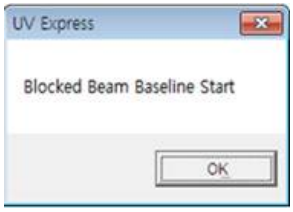
NOTE: *You can save the method and open the method whenever you need.*
ex) Save [File] → [Save Method] and Open [File] → [Open Method]

6. Input the blank solution into Reference and Sample holder both. Close the sample compartment cover and measure Baseline using **Baseline** icon. Absorbance of selected wavelength is changed about 0 AU.

NOTE: *When you check **the 0%T / Blocked Beam Baseline**, Baseline measurement procedure is as follows.*

a. Click **Baseline** *icon.*

b. *When the below message is popped up, click* ***OK*** *after checking the empty of both holders (Reference and Sample).*

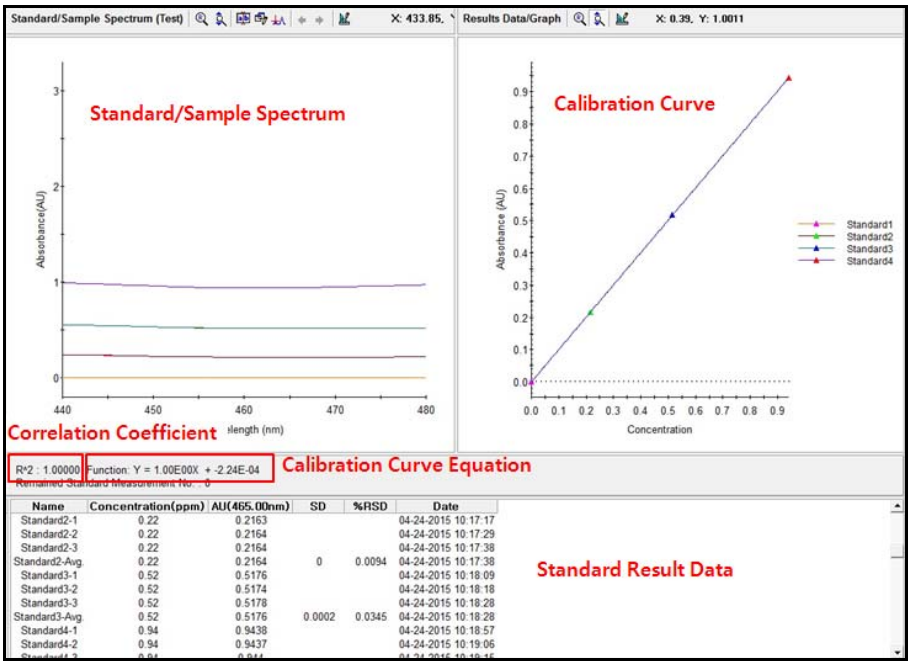


c. *Measure the dark automatically and then, after the below message is shown. Depending on the sample, empty the cell holder or input the blank into reference and sample holder both. Click* ***OK****.*

NOTE: *You should measure Baseline whenever the wavelength, SBW or Reference sample is changed*.

7. Input the Standard sample into the Sample holder and then, select the **Standard** icon.

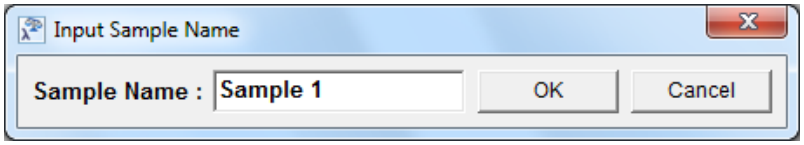
8. Measure Standard samples in order and the calibration curve will be created as follow.



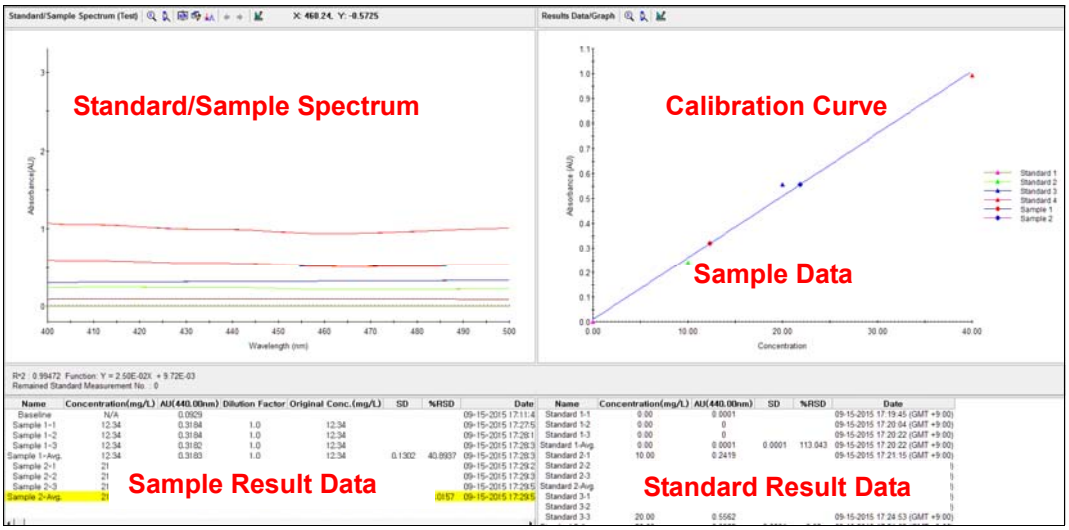
NOTE: *You can save the standard calibration curve and open the curve whenever you need.*

9. Input Blank and Unknown sample into each Reference and Sample holder and then, click **Sample** icon. Click **Sample** after measuring [Baseline] when you opened the standard curve saved.

10. Input sample name and select **OK**.



11. Data will be shown as follows.



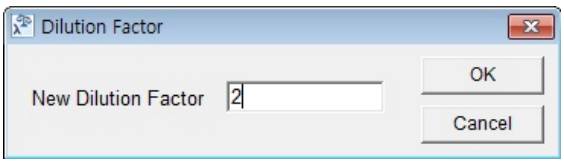
a. Name: Shows the entered Standard or Sample name.

b. Type: Displays the Standard or Sample.

c. Concentration: Display the calculated concentration.

d. AU: Displays the measured Y-axis value.

e. Dilution factor: The dilution factor is set 1.0 as a default and it can be edited after the sample measurement. Double-click on the Dilution Factor to be changed in each sample measurement result and enter a new Dilution Factor and click **OK**.

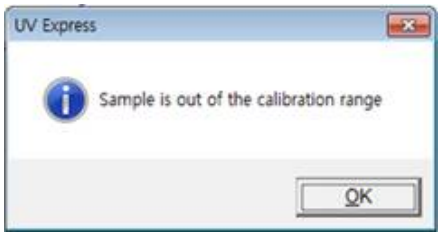


f. Original Conc.: Shows the original concentration of sample.

(Original Concentration = Concentration x Dilution Factor)

g. SD, %RSD: Shows the statistics of the repeated measures data.

NOTE: *In case of checking on Limit warning Message in the Quantification tab of the Method window, the following message is shown as the sample measurement values is out of the calibration curve range.*



12. Save or print spectrum and results as desired.

XI. Display Function Tools

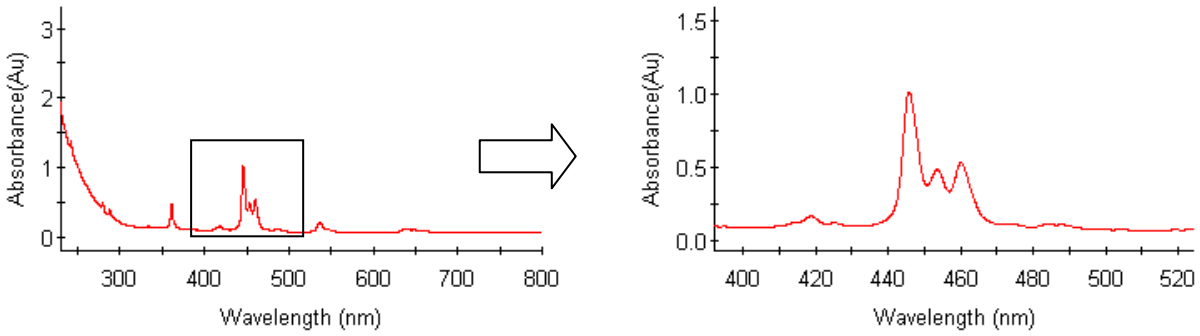
Use the display function tools to modify the display of the main window as desired.

Tool icon	Function	Tool icon	Function
	Zoom Reset		Pick Peak
	Y Axis Auto Scale		Pick Valley
	Add Label		Cursor
	Edit Label		To Left
	Properties		To Right

XI-1. Zoom In / Reset / Auto scale

- Use these icons to zoom, reset and auto scale the selected area in the main window.
- Procedure

1. Select the zoom area using the mouse as shown below.



2. To restore the original range, click **Zoom Reset** or double click anywhere in **Main Window**.

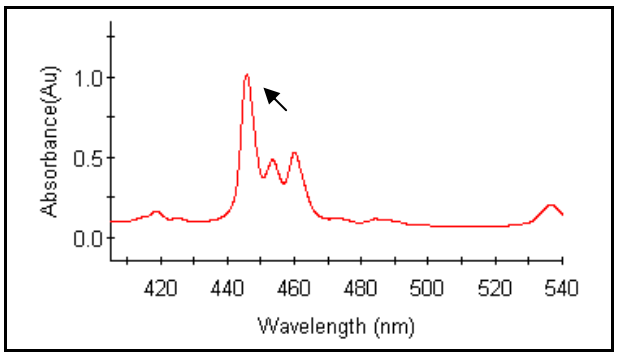
3. To Auto scale the Y axis, click the **Y Axis Auto Scale** .

XI-2. Add / Edit / Delete Label

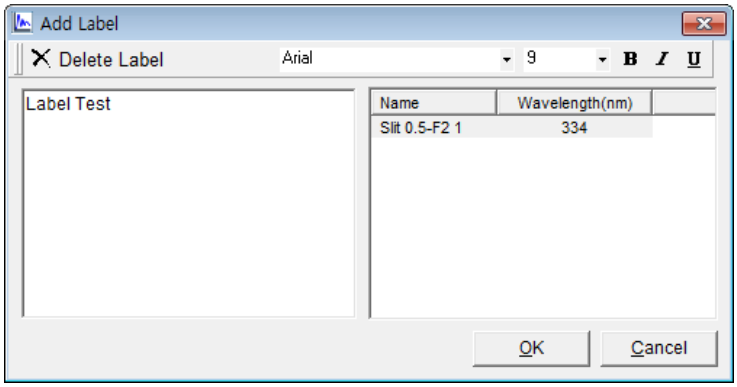
- Use these icons to add, edit and delete labels in the main window.
- Procedure

1. Click **Add Label** .

2. Set and click the label at the required position using the mouse as shown below.

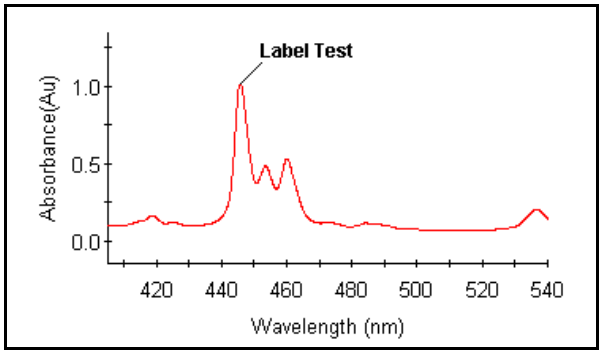


3. The following dialog box will be displayed.



4. Enter comments, and select a font style and size.

5. Select **OK**.



6. To edit or delete the labels, click **Edit Label** and edit or delete the labels as desired.

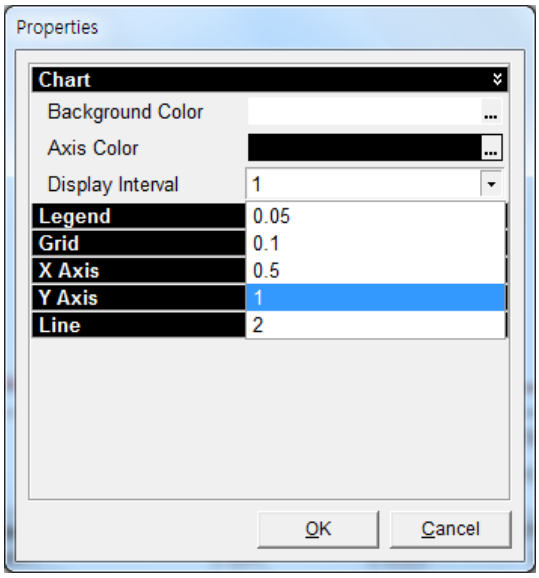
XI-3. Properties

- Use these icons to change chart properties in the main window, such as the chart color, display interval, grid, X Axis, Y Axis and line properties.
- Procedure

1. Select **Properties**.

2. Change properties as follows.

3. Select **OK** when finished.



a. **Chart**: Choose the colors of the background and Axis color.

Background Color: Select the main window color.

Axis Color: Select the spectra axis color.

Display Interval: Select the wavelength display interval: 0.05 nm, 0.1 nm, 0.5 nm, 1 nm, 2 nm.

b. **Legend**: Select to display the legend on the chart and where to position it.

Show: Select Yes or No.

Location: Select the legend position; Top, Bottom, Left or Right.

c. **Grid**: Select to display X and Y grids.

Pattern: Select a grid pattern; None, Solid, Long Dash, Dotted or Short Dash.

Width: Set the grid width.

Color: Select grid color.

X Space: Set the space of X-axis.

Y Space: Set the space of Y-axis.

d. **X Axis**: Set the range of X Axis.

Min. and Max.: Set the range of X-axis.

Decimal Point Format: Set the decimal point (1~9).

e. **Y Axis**: Set the range of Y Axis.

Auto Scale: Select Yes or No.

Min. and Max.: Set the range of Y-axis

Decimal Point Format: Set the decimal point (1~9).

f. **Line**: Select the color and pattern of the spectrum lines.

Pattern: Select a grid pattern; None, Solid, Long Dash, Dotted or Short Dash.

Width: Set the line width.

Color: Select line color.

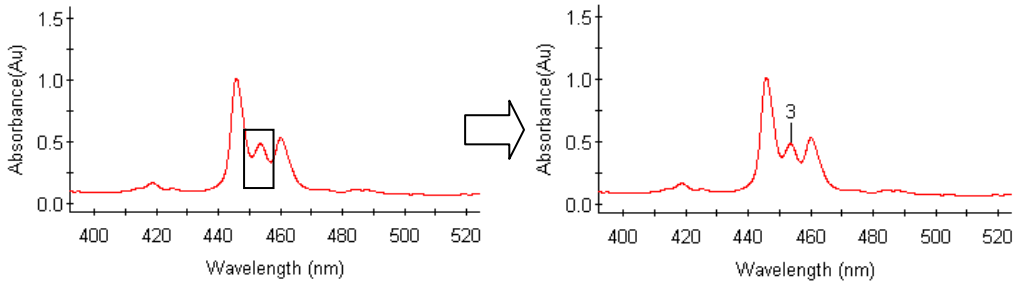
XI-4. Pick Peak / Valley

- Use these icons to pick peaks/ valleys or seek the data.
- Procedure

1. Select **Pick Peak/ Valley**.

2. Select the spectral range using the mouse. The labels of the peaks or valleys are displayed as shown below.

3. To delete a peak/valley, select the peak/valley in the result window and select **the right mouse.**



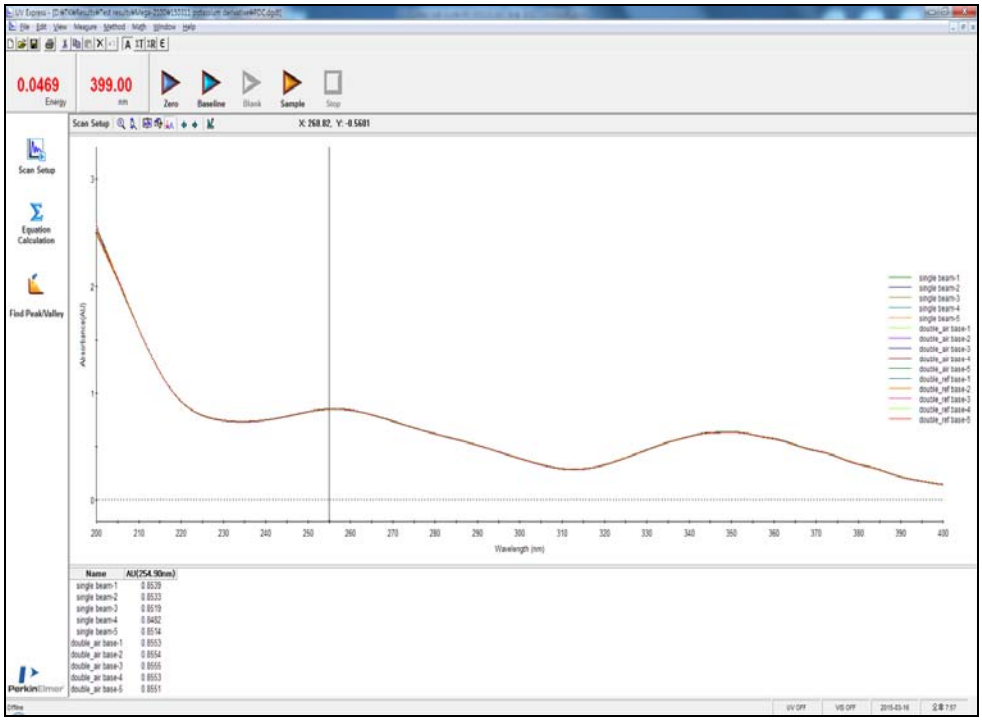
4. Select **delete** or **delete all** as shown below.

Name	No.	Peak(nm)	Peak(AU)
Sample1	1	241.20	0.3829
	2	250.00	0.1165
	3	278.20	0.3274
	4	287.40	0.3597
	5	333.50	0.1651

Delete
DeleteAll
Copy

XI-5. Cursor / To left / To right

- Use these icons to display the cross lines for selecting data points.
- Procedure

1. Select **Cursor** .

2. Wavelength and absorbance (Transmittance or reflectance) value will display in the result window. User can move the cursor using To left or To right icon.