UV-VIS SPECTROSCOPY

LAMBDA 365 Multi-Component Analysis

Software Guide

PerkinElmer

Release History

Part Number	Release	Software Version	Publication Date
09931304	D	Multi-Component Analysis SW 4.0.1	September 2016

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I. Introduction

I-1. Overview

Multi-Component Analysis (MCA) software is to analyze complex compounds containing several compounds. Up to 4 components in the mixture can be analysed. It is used to define the concentration of each component.

This manual provides step-by-step instructions for the use of UV Express Multi-Component Analysis software for a Lambda 365 UV-Vis spectrophotometer.

Multi-Component Analysis software must be installed onto a Microsoft® Windows 7 or Window 8 operating system to function properly.

I-2. Minimum PC Requirements

Operating Environment

Support Double-beam UV-Vis Spectrophotometer

· Lambda 365 UV-Visible Spectrophotometer

Minimum Computer Requirements

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

Operating System

· Microsoft® Windows 7

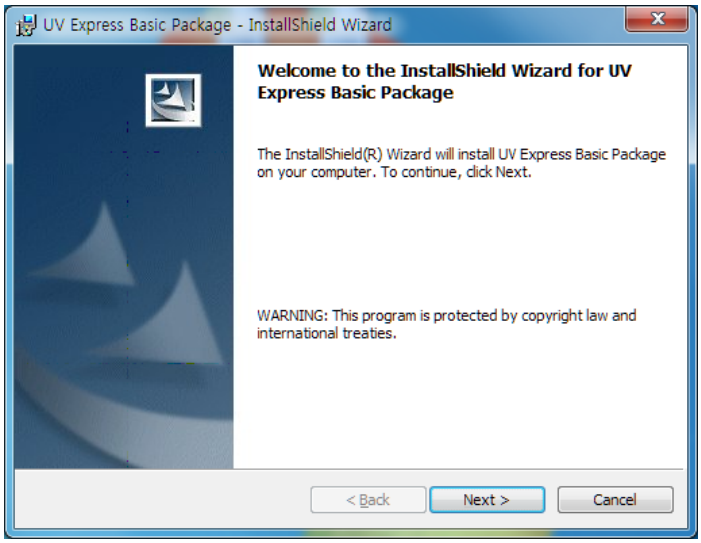
Output Device

· Microsoft® Windows compatible printer

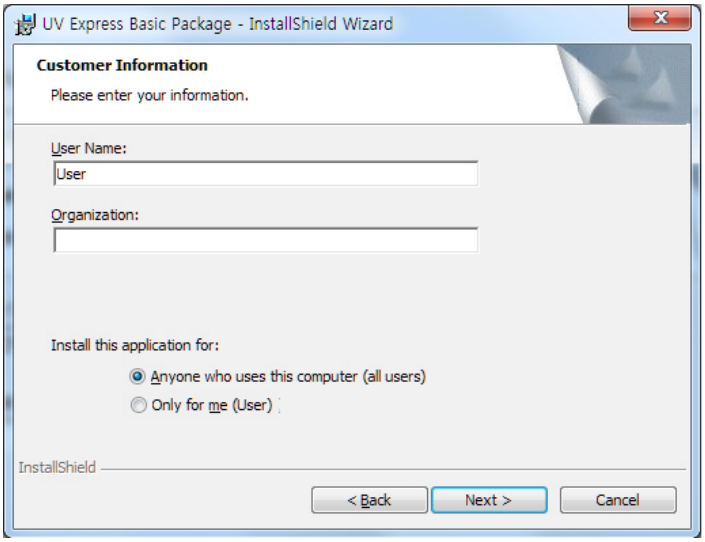
I-3. Multi-Component Analysis Software Installation

NOTE: *The UV Express software should be installed prior to installing the Multi-Component Analysis software*

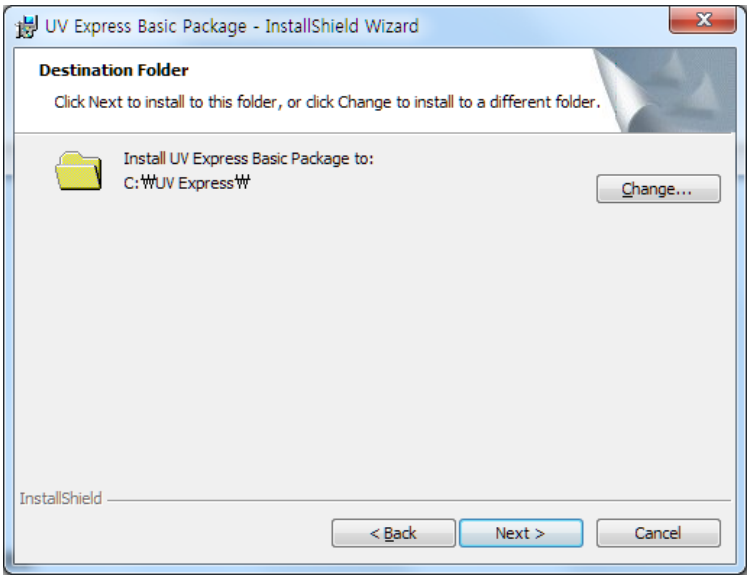
1. Input the **UV Express software** CD into the CD drive. UV Express software is installed automatically. The default installation folder is "C:\UV Express".
2. If the automatic setup window is not shown, click **Setup.exe** in the CD.
3. UV Express InstallShield® Wizard starts. Click **Next**.



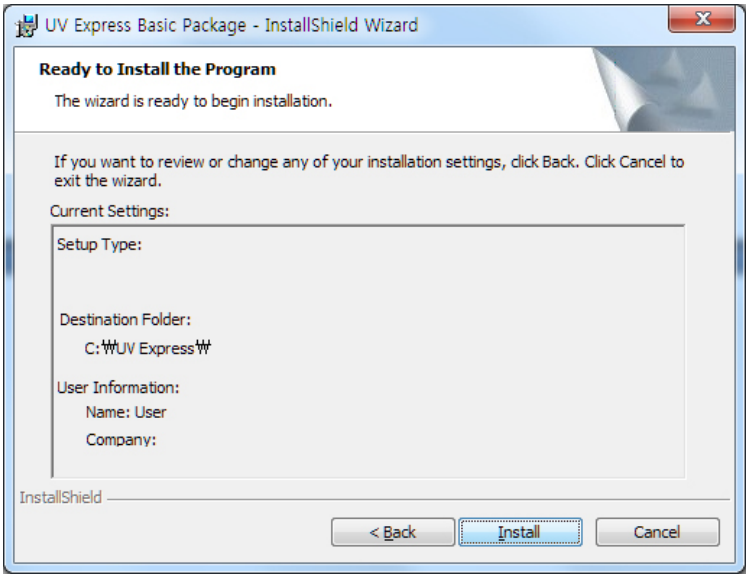
4. Enter your information and click **Next**.



5. Click **Next**.



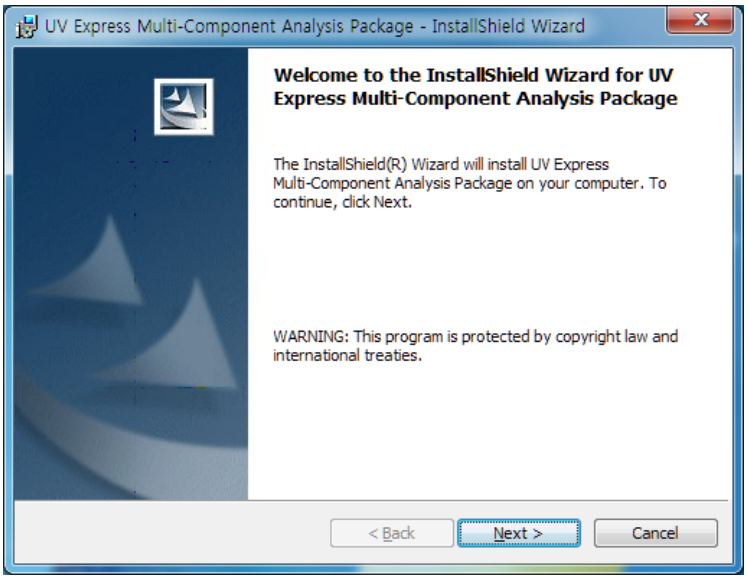
6. Click **Install**.



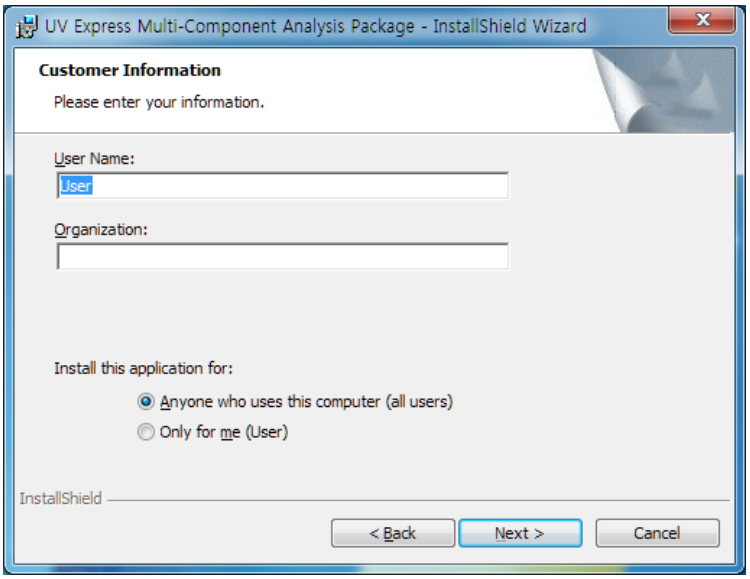
7. After the set up is complete successfully, click **Finish**.

8. After the UV Express Software installation is complete, the UV Express folder  will be created on the Desktop.
9. Input the **UV Express Multi-Component Analysis software** CD into the CD drive. UV Express Multi-Component Analysis software is installed automatically. The default installation folder is "C:\UV Express".
10. If the automatic setup window is not shown, click **Setup.exe** in the CD.

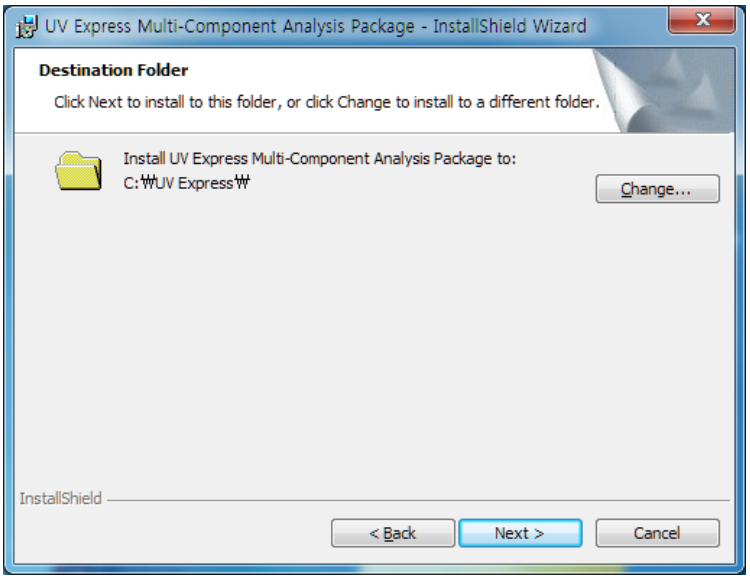
11. Multi-Component Analysis InstallShield® Wizard starts. Click **Next**.



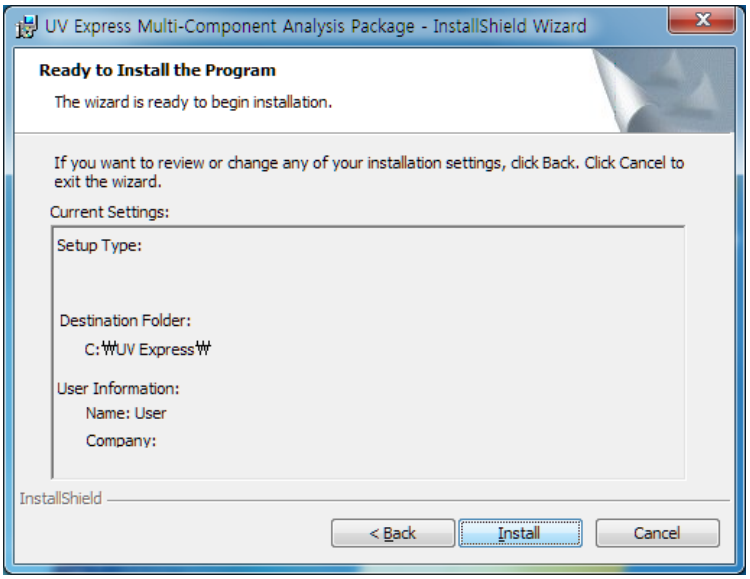
12. Enter user information and click **Next**.



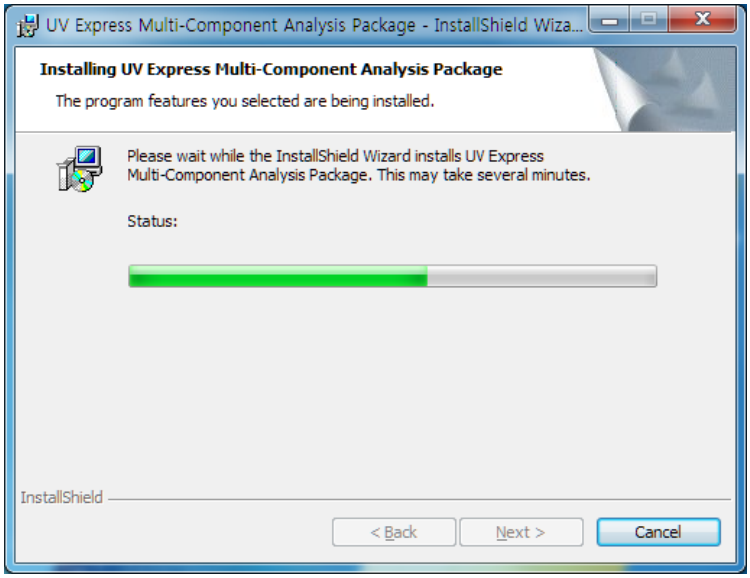
13. Click **Next**.



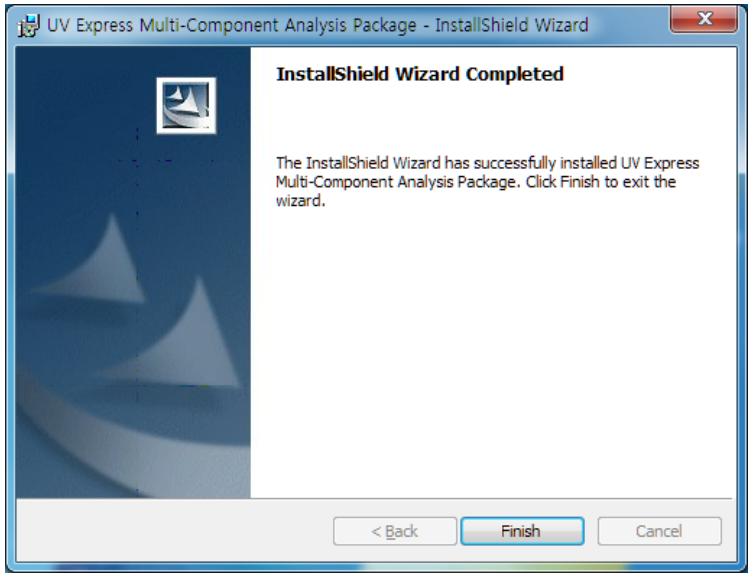
14. Verify the current settings and click **Install**.



15. The following window shows the status of the Multi-Component Analysis software installation.

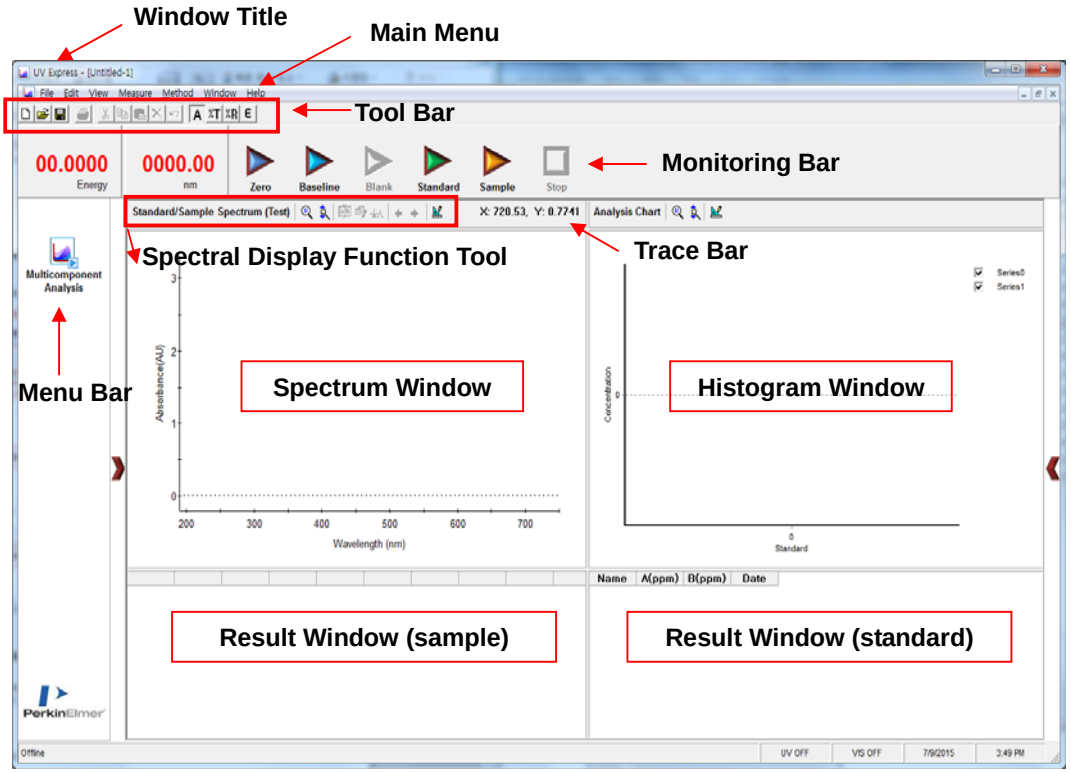


16. After the setup is completed successfully, click **Finish**.



I-4. Multi-Component Analysis Software Interface

This chapter describes the unique display features of Multi-Component Analysis Software.

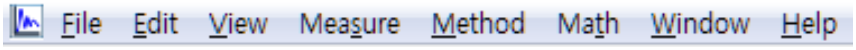


I-4-1. Window Title

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-4-2. 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.



NOTE: *For more details of the Main Menu, refer to the UV Express Software Users Guide.*

I-4-3. 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-4-4. 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
	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.

I-4-5. Spectrum window

Display the spectrum of performed standards and samples measurements.

I-4-6. Histogram window

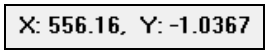
Display the estimated sample concentrations from standards.

I-4-7. Result window

Display result values of performed measurements.

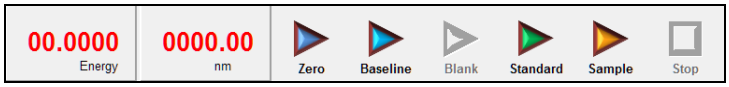
I-4-8. Trace bar

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



I-4-9. Monitoring Bar

Display the current photometric value, wavelength and icons 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
	Collects a standard spectrum
	Collects a sample spectrum
	Stops measuring data

II. Operation

▣ Multi-Component Analysis mode allows to quantify the unknown mixed samples' concentration based on standards at the specified wavelength or range among one of the measured spectral range.

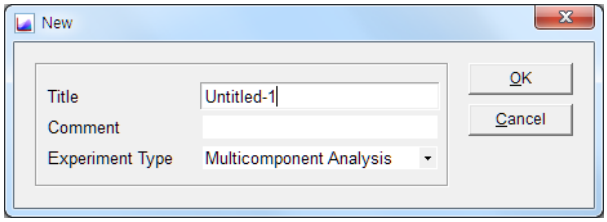
▣ Use Multi-Component Analysis mode to quantify a sample at one of the scanned wavelength using a reference standard.

▣ Perform a Multi-Component Analysis Method measurement as follows:

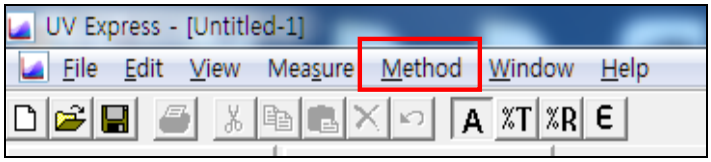
1. Set method parameters.
2. Measure Baseline.
3. Scan with standards for estimation.
4. Scan samples.
5. Save or print results as required.

II-1. Multi-Component Analysis Setup

1. Execute the **Multicomponent Analysis** .
2. The following window will be shown. Enter **Title, Comment** and select **OK**.

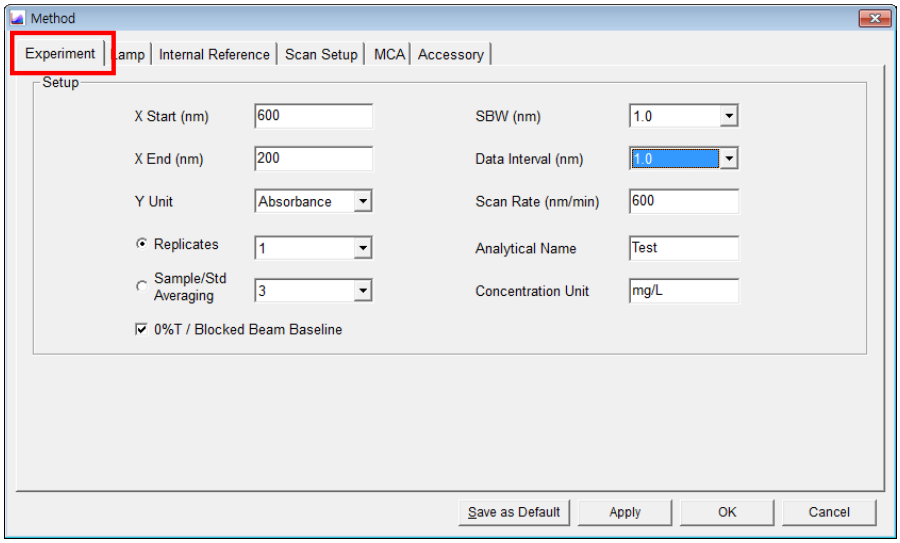


3. Select **Method**.



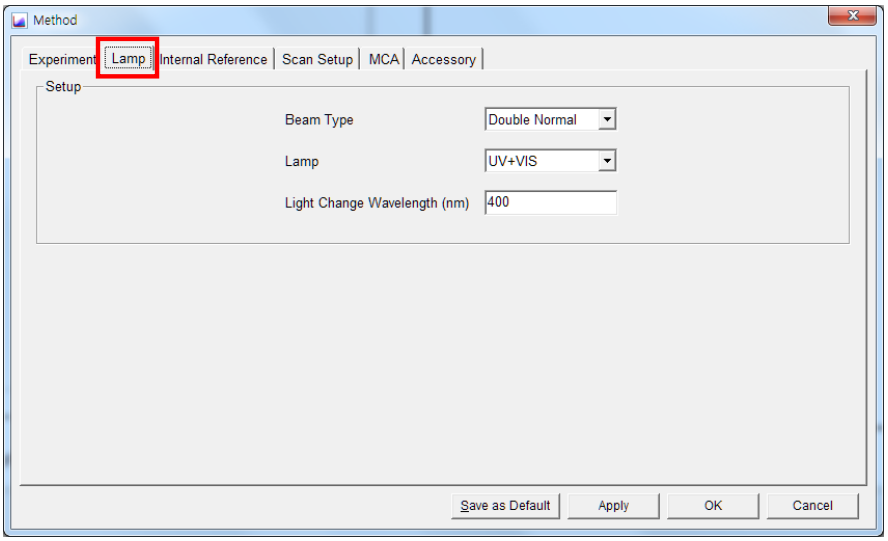
4. Setup the experiment parameters as follows:

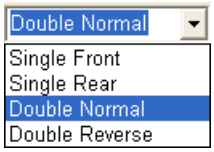
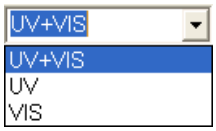
4.1 Experiment



Command	Function
X Start (nm)	Input the start measurement wavelength (190 to 1100 nm)
X End (nm)	Input the end measurement wavelength (190 to 1100 nm)
Y Unit	Displays Y-axis unit. Absorbance (AU), Transmittance (%T), Reflectance (%R) or Energy
Replicates	Select the number of repeated readings to be taken for each sample (1~5)
Sample/Std Averaging	Select the number of sample/standards to be taken and averaged (1~3)
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, 1.0, 2.0, 5.0 or 20.0 nm. 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)	Enter the scan speed
Analytical Name	Enter the analytical name
Concentration Unit	Enter the concentration unit

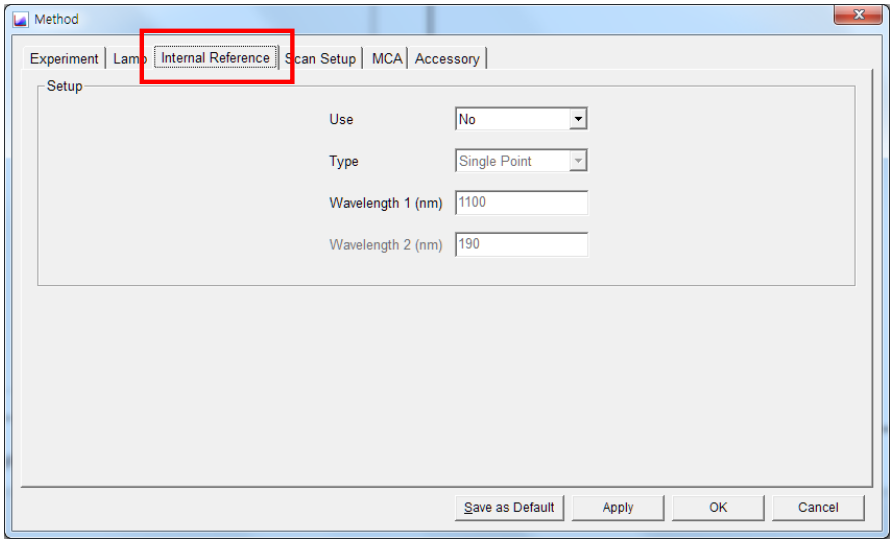
4.2 Lamp

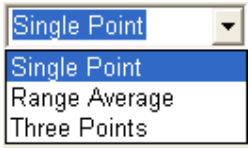


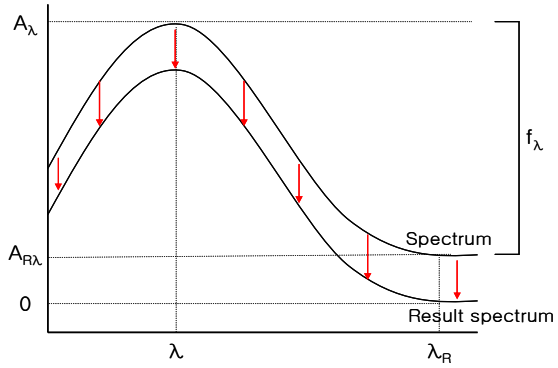
Command	Function
Beam Type	Select Beam Path Type.  Single Front: Only uses Sample holder as a single beam type Single Rear: Only uses Reference holder as a single beam type Double Normal: General uses as a double beam type Double Reverse: Reverses the sample and reference position as a double beam type
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 whether user will use the Internal Reference or not.
	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

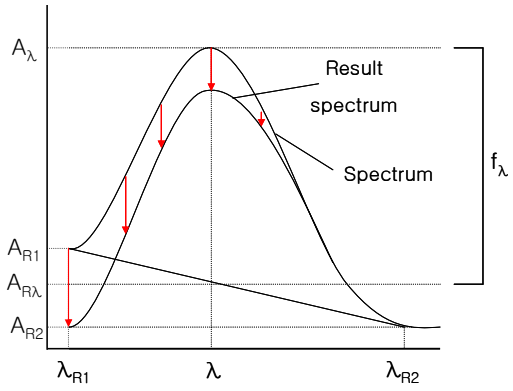
Type

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.

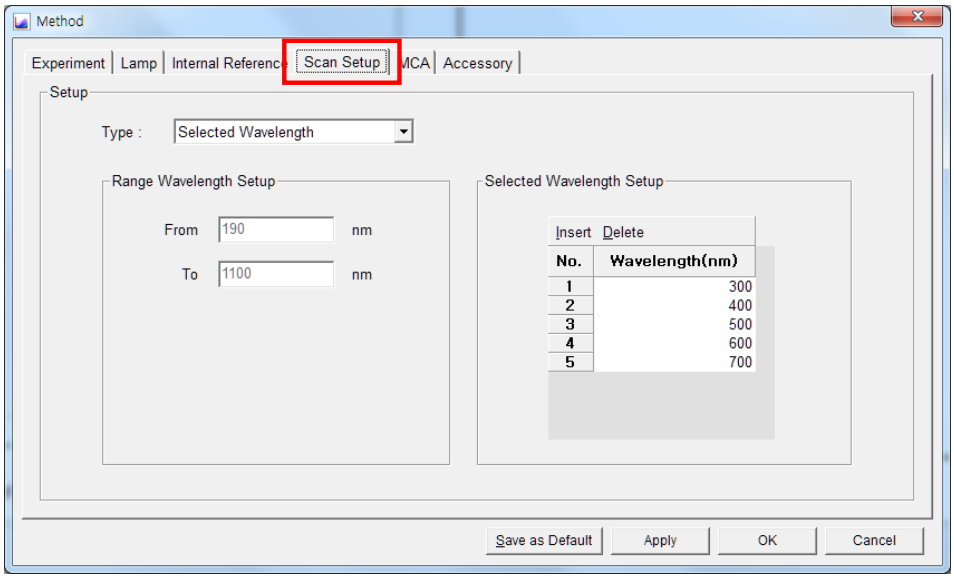
Three Points:

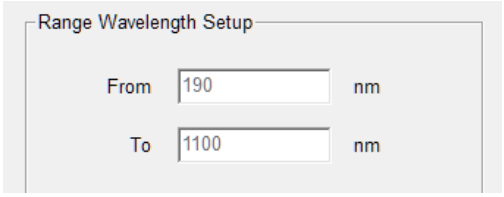
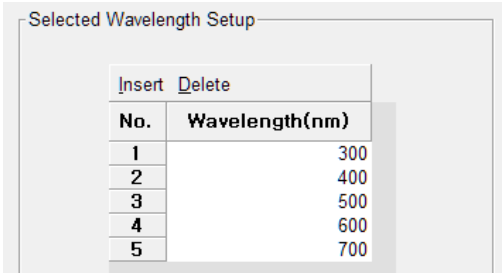
Three points method is used correction of slant baseline, then the absorbance values from the two reference wavelengths, A_{R1} and A_{R2}, define a straight line which is used to calculate the reference absorbance ($A_{R\lambda}$) at the wavelength(λ). Result spectrum calculated by following equation.



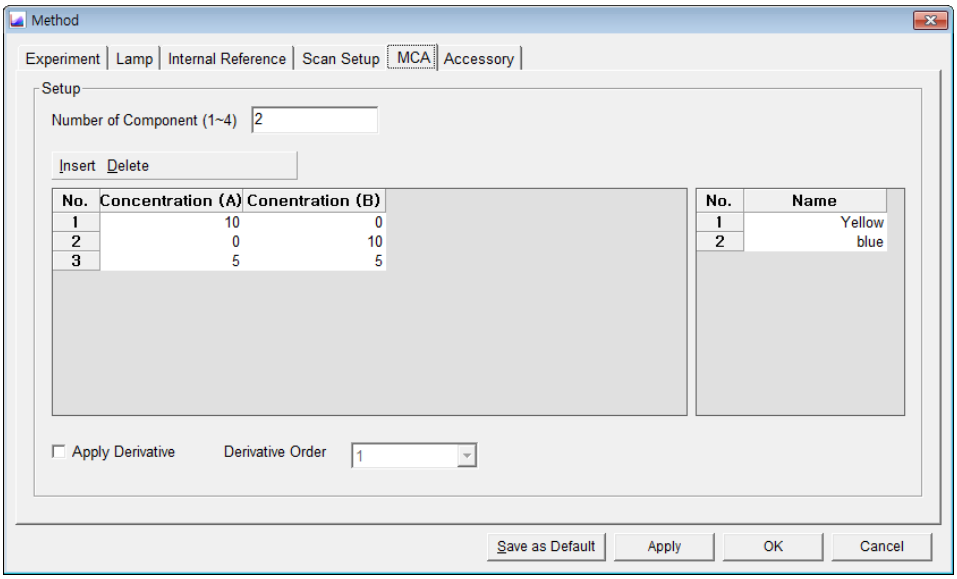
	$$A_{R\lambda} = \frac{1}{\lambda_{R2} - \lambda_{R1}} \{(\lambda_{R2} - \lambda)A_{R1} + (\lambda - \lambda_{R1})A_{R2}\}$$ $f_\lambda = A_\lambda - A_{R\lambda}$ f_λ is the function result at wavelength λ A_λ is the absorbance at wavelength λ $A_{R\lambda}$ is the reference absorbance at wavelength 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.

4.4 Scan Setup



Command	Function
Type	Select a calculation method for MCA. (Range Wavelength or Selected Wavelength)
Range Wavelength Setup	If Range Wavelength is selected in Type, the concentration of sample is estimated using the specified range of standard spectrum. 
Selected Wavelength Setup	If Selected Wavelength is selected in Type, the concentration of sample is estimated using the specified points of standard spectrum. 

4.5 MCA



Command	Function
Number of Standard (1~4)	Enter the number of standards to be measured.
Standard Parameter Setting	Enter the name and concentration of each standard in the text box. **Insert** and **Delete** can be used to change the number of standards for the test. 
Apply Derivative	Check (√) in order to obtain the data with derivative applied. Enter the derivative order number (1~4) once checked (√).

4.6 Accessory

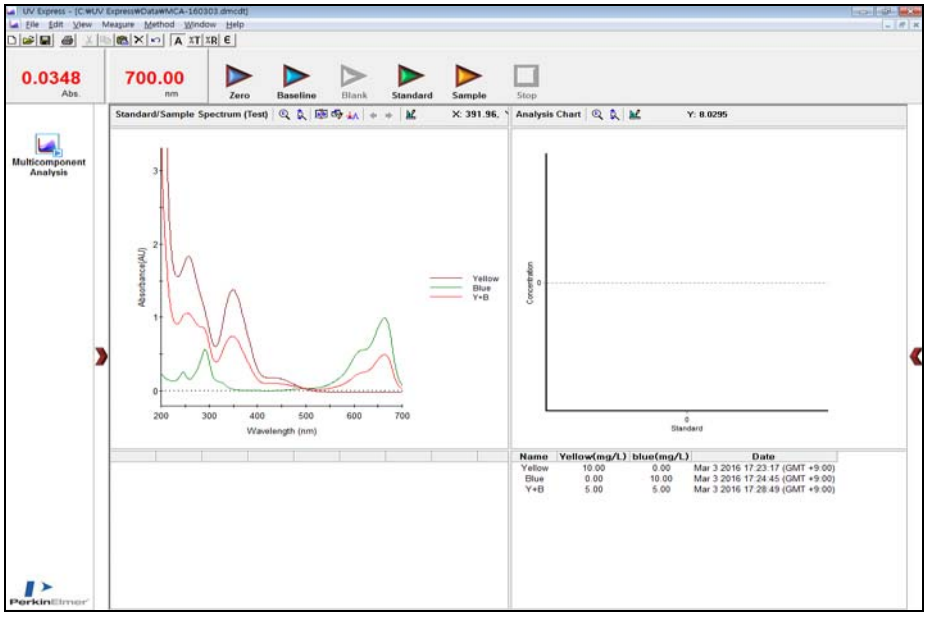
For more details of accessory setting, refer to each accessory manual.

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 clicking **Baseline**  icon.

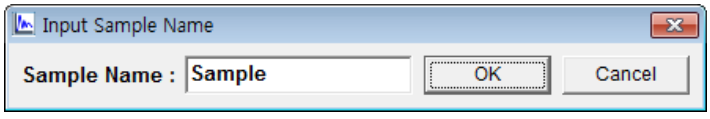
NOTE: *The baseline defines the absorbance 0 (or transmittance 100%) and it is subtracted from the measurement result to obtain a correct sample spectrum (or it is divided in the case of transmittance).*

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

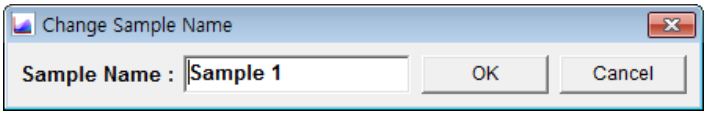
7. Input the Standard sample into the Sample holder and then, click the **Standard** icon.
8. Measure each Standard sample in order and Standard spectrum result will be depicted as follows.



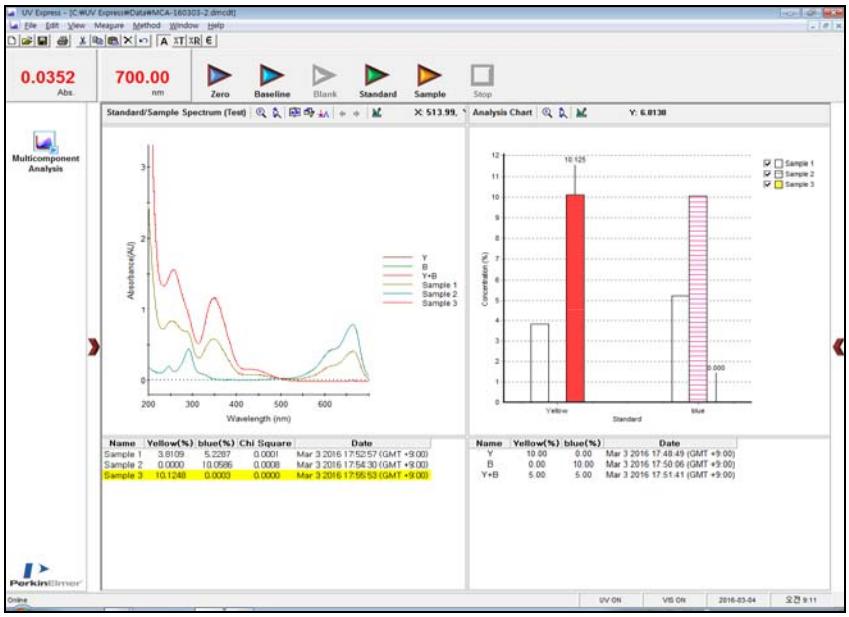
9. Input the sample into the sample holder and close the sample compartment cover and then, click **Sample** icon.
10. Input the sample name and click **OK**.



NOTE: *After measuring sample, if you want to change the sample name, double click on the result line in the Result Window. Then the selected line's color will be changed yellow.*
*Type a new name and click **OK**.*



11. The spectrum and result will be shown as below.



12. Contents of each standard component in the unknown sample and its Chi Square value are displayed.

13. Save or print spectrum and results as desired.

? *Chi-square, χ^2*

The Chi-square distribution is used in the following cases;

a. to examine the discrepancy between an observed frequency and an expected frequency when more two results are acquired

b. to examine whether the sample distribution corresponds to a binomial distribution or a normal distribution

c. to examine whether two variables are independent each of other or not.

The following statistic can be used as a tool to measure the discrepancy between observed frequency and expected frequency:

$$\chi^2 = \frac{(o_1 - e_1)^2}{e_1} + \frac{(o_2 - e_2)^2}{e_2} + \ldots + \frac{(o_k - e_k)^2}{e_k} \equiv \sum_{i=1}^{k} \frac{(o_i - e_i)^2}{e_i} \quad \ldots\ldots\ldots (1)$$

If the total frequency is "N", the following equation is formulated:

$$\sum o_i = \sum e_i = N \quad \ldots\ldots(2)$$

The former equation can be also expressed as:

$$\chi^2 = \sum \frac{o_i^2}{e_i} - N \quad \ldots\ldots(3)$$

if $\chi^2 = 0$, the observed frequency corresponds to the expected frequency. exactly,

if $\chi^2 > 0$, they do not correspond exactly. That is, the larger the value of χ^2, the larger discrepancy between the observed frequency and the expected frequency.