

## **Micro-Volume UV Spectrophotometer**

## Model No: YSA-400

# **User Manual**

Version: V1.00 Software Version: V1.00 Hardware Version: V1.00



Yu-Shing Biotech., Ltd

## Declaration

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Now, enjoy the convenience of YSA-400 micro-spectrophotometer if you obey the rules.

#### Package List

Every product has following objects. They should be checked carefully when you open the package for the first time. Yu-Shing Biotech., Ltd. and the dealers will replace relative articles for free if they are found different from the ones in the following table.

Name	Piece	Remark
YSA-400 micro-spectrophotometer	1	200-850nm
The CD for YSA-400	1	200-850nm
USB connector	1	
12V Power adapter	1	
12V Power adapter connector	1	

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### **1** Introduction

The YSA-400 micro-spectrophotometer, a new type full-spectrum(200  $\sim$  850nm) spectrophotometer, can measure 0.5  $\sim$  2 µL samples with high accuracy and reproducibility. The samples can be measured by dropping them directly onto the lower measurement pedestal without absorption cell and also can be taken back after measuring if necessary. This instrument don't need warm-up after start, and is operated simply and quickly with reporting directly the concentrations of samples. The concentration range of samples measured by K5500 micro- spectrophotometer is 50 times larger than that measured by the general spectrophotometer. In addition, the instrument is small (outline size: 200mm×170mm×110mm) and light (net weight: 1.35kg).

#### Principle

The calculation of sample concentration is based on the Beer-Lambert law,

#### $A = \varepsilon b c$

Where *A* is the measured absorbance,  $\varepsilon$  is the molar absorption coefficient, *b* is the path length, and *c* is the concentration.

#### Range of application

The YSA-400 micro-spectrophotometer can measure:

**Nucleic acid:** the concentration and purity of DS-DNA, SS-DNA and RNA. **Protein:** ①the concentration of the protein (1Abs = 1mg/mL), BSA, IgG and lysozyme; ②the concentration of protein with protein assay kit (Lowry, BCA and Bradford), and the software can automatically create the standard curve and report directly the concentration of the sample.

**UV/VIS:** the UV/VIS full-spectrum (200 $\sim$ 850nm) absorbance scan.

Cell Culture: the concentration of suspended cell cultures.

**Micro Array:** the concentration of fluorescent dye labeled nucleic acid (micro array), for it can measure the concentration of the nucleic acid and the dye at the same time.

## 2 Installation

#### 2.1 Port connection

Connect the USB port of the YSA-400 micro-spectrophotometer to the USB port of computer with the USB connector.

Connect the adapter to the power plug with the power adapter connector. Plug the plug of the adapter into the jack of the YSA-400 microspectrophotometer.

#### 2.2 System requirements

Software requirements:	Microsoft Windows 2000 and XP or better operation
	system
	Microsoft Office 2000 or higher office software
	Adobe Reader 7.0 or higher version
Hardware requirements:	CPU: Intel Pentium III 800MHz or above
	EMS memory: a minimum of 256MB
	Hard disk: with a minimum of 50MB of free disk
	space

#### 2.3 Installing the software

Put the YSA-400 software CD into the CD drive. Double-click the CD catalogue, open the "Setup" folder and run the "YSA-400 Install.exe" file to start the installation process of the software.

Click "Next", choose installation catalog as below:



Click "Next" and input the serial number.

Click "Next" to start the installation of the software.

Click "Close" when installation is finished.

The YSA-400 icon will automatically be formed on the desk.



#### 2.4 Installing the drivers

After the instrument is connected correctly with the computer, Windows will automatically scan the new hardware and tip: Find new hardware.

Windows will automatically open hardware installation guide. Now select "Install from a list or specific location (Advanced)" as below:



Click "Next" and enter driver options dialog box. Click "Don't search, I will choose the drivers to install" as below:

尋找新增硬體精靈		
<b>若靈搜尋中,諸稍候</b>		
Ocean Optics USB2000+	S	
	<上一步(B) 下一步(N) > (	取消

Wait, Windows system will automatically install the selected divers. When the installation is finished, a dialog box will appear as below:



Click "OK" to finish the hardware installation.

Now, Windows will tip you that the instrument is installed successfully and can be used.

## **3 General operation**

#### 3.1 Sample size requirements

Nucleic acids:	0.5~2.0µL
Proteins:	0.5~2.0µL
Lowry, BCA and Bradford:	0.5~2.0µL
Cell suspensions:	0.5~2.0µL
Other solutions:	0.5~2.0µL

#### 3.2 Measurement procedures

3.2.1 Start

(1) Connect correctly YSA-400 micro-spectrophotometer to the computer.

(2) Connect correctly YSA-400 micro-spectrophotometer to the power adapter.

(3) Switch on the power by pressing the switch, then the red light will come on.

(4) Make sure the sampling arm is closed and the pedestals are clean, or it will seriously impact the measurement result.

(5) Double-click the K5500.exe icon on the desk or one-click the K5500.exe icon from Start menu to start the K5500 software.



(6) Then the instrument starts self-checking.

(7) If the following box pop up, please press OK and check whether the instrument is connected correctly to the computer, the sampling arm is closed and the pedestals are clean. Then you must switch off the power of the instrument by pressing the switch and repeat (1)-(7).



(8) The above picture will disappear and the work interface will open as below when the self-checking finished.



Now you can measure the sample from here.

#### 3.2.2 Blank

(1) You must choose the correct measurement type from the measurement type select area based on you samples before measuring.

(2) A blank must be measured before measuring the samples or after reselecting a measurement type each time.

(3) Then open the sampling arm and drop  $0.5 \sim 2\mu L$  solvent onto the lower measurement pedestal.





(4) Close the sampling arm and click the "Blank" button. The sample column is automatically formed as below and the software will automatically measure and store the result of the blank.



(5) Open the sampling arm and wipe the blank solvent from the measurement pedestals with clear absorbent paper.

#### 3.2.3 Measurement

(1) Open the sampling arm and drop  $0.5\,{\sim}\,2\mu L$  sample onto the lower measurement pedestal.

(2) Close the sampling arm and click the "Measure" button, and the software will automatically measure the sample.

(3) The result (values and graph) of the sample will appear in the work interface in seconds.

If you aren't sure about the result or you don't think the curve is normal, the reasons may be that the sample column isn't formed or the blank doesn't work well. Please check whether the sample column is formed and measure it again, or measure the blank again and then measure the sample again.

(4) Click the "Save Graphic" button after measured a sample if you want to save the graph.

(5) Open the sampling arm and wipe the sample from the measurement pedestals with clear absorbent paper after each time a sample measured.

3.2.4 Output

Click the "Show Report" button after the samples measured to output the result data of the samples by excel.

### 4 Functions of work interface

Open the YSA-400 software and enter the work interface of the YSA-400 micro-spectrophotometer as below:



For easy introduction, the work interface can be divided into five parts: menu bar, measurement type select area, graph display area, data display area and measurement function area.

#### 4.1 Menu bar

There are four main menus in the menu bar: "File", "View", "Tool" and "Help".

File(F) View(V) Tool(T) Help(H)

**File:** An "Exit" menu is under the "File", and the program will exit if click the "Exit".



**View:** There are four submenus under the "View": "Auto Scale", "Multi Draw", "Show Grid" and "Color Solution".

File(F)	View (V)	Tool (I)	Help (H)
Nucl	<u>A</u> uto S	Scale	Ctrl+A
NUCH	Multi Draw		Ctrl+M
	✓ Show @	Frid	Ctrl+G
	Color	Configure	Ctrl+C

"Auto Scale" submenu enables the user to scale the Y axis. The default setting can automatically scale the Y axis based on the sample. The user may also input the max value of the Y axis from the "Auto Scale Input" dialogue which will appear when click the "Auto Scale".

AutoScale Input	×
Max value o	of Y axis:
10.0	
Set	Cancel

"Multi Draw" can let several scan curves resulted from several measurements appear in the graph display area at the same time. So the user can compare the reproducibility of the same sample or the changes of different samples. "Show Grid" can let the back ground grid appear or cancel.



Click "Color Solution", a "Form Color Solution" dialog box will appear. From here the user can change the colures of graph display area, such as the background, the measurement lines and the scan curves. And the color of measurement lines and scan curves should be different from the color of background.

Back Ground	CIBlack	•
Grid	clSilver	•
Axis	CIBlack	•
Number	CIBlack	•
Xa	clFuchsia	•
×ь	cfYellow	•
Xc	📘 clAqua	•

**Tool:** The "Check Calibration" and "Dye Editor" submenus are under the "Tool".

File(F) View(V)	Tool (T)	Help(H)
	Check	Calibration Ctrl+C
NUCIEIC AC	<u>D</u> ye Ed	ditor Ctrl+D

Click "Check Calibration" to pop out a "Check Calibration" dialog box which enables the user to check whether the light intensity is appropriate.

The light intensity has been adjusted to the perfect condition before leaving factory. Clicking the UV or VIS will check the light intensity and produce curves of light intensity.



"Dye Editor" enables the user to save the information of fluorescent dyes from the table as below:

Name	1/M-cm	nm	260nm%	280nm%	Save & Exit
СуЗ	150000.000000	550	0.040000	0.050000	0.000
Cy5	250000.000000	650	0.000000	0.050000	Exit
Alexa Fluor 488	710000.000000	495	0.300000	0.110000	
Alexa Fluor 546	104000.000000	556	0.210000	0.120000	
Alexa Fluor 555	150000.000000	555	0.040000	0.080000	
Alexa Fluor 594	73000.000000	590	0.430000	0.560000	
Alexa Fluor 647	239000.000000	650	0.000000	0.030000	
Alexa Fluor 660	132000.000000	663	0.000000	0.100000	
Су3.5	150000.000000	581	0.080000	0.240000	
Cy5.5	250000.000000	675	0.050000	0.180000	
2					

The respective extinction coefficient, absorbance wavelength and 260nm and 280nm % corrections are shown in the table.

**Help:** The "Help" menu will help the user understand the majority of questions met during using the software. There are "About" and "Help" submenus under the "Help" menu.

File(F) View(V) Too	1 ( <u>T</u> )	Help (H)	
Nuclaia Aaid R		About	Ctrl+B
NUCIEIC ACIU	FI	Help	Ctrl+H

And clicking the "Help" submenu will open YSA-400 micro-spectrophotometer user manual.

#### 4.2 Measurement type select area

There are six items in the measurement type select area: "Nucleic Acid", "Protein", "UV-VIS", "Cell Culture", "Micro Array" and "Sample &Test Type", and "Sample &Test Type" is the supplement for the former five items. The user can select the correct measurement type from here based on the samples.

```
Nucleic Acid Protein UV-VIS Cell Culture MicroArray
```

**Nucleic Acid:** This type is designed for measuring the concentration and purity of nucleic acid. The user can select the "DS-DNA (50)", "SS-DNA(33)" or "RNA(44)" under the "Sample &Test Type" to measure the double-stranded DNA, the single-stranded DNA and RNA respectively.



**Protein:** This type is designed for measuring the concentration of protein. Click the "Protein" button and enter the protein measurement interface. The user can select a specific type from the "Sample &Test Type" where are eight types: "A280(1)", "BSA(6.7)", "IgG(13.7)", "Lysozyme(26.4)", "Labels", "BCA", "Bradford" and "Lowry".

Sample&Test Ty	pe
A280(1)	-
A280(1)	
BSA(6.7)	
lgG(13.7)	
Lysozyme(26.4)	
Labels	
BCA	
Bradford	
Lowry	

"A280 (1)" is designed for measuring the protein solution which produce an absorbance at 280nm of 1.0A (path length: 10mm) at the concentration of 1 mg/ml.

"BSA(6.7)", "IgG(13.7)" and "Lysozyme(26.4)" are designed for measuring the BSA, IgG and Lysozyme.

"BCA", "Bradford" and "Lowry" are designed for measuring the concentration of proteins with protein assay kit. A program designed by the software can automatically generate the standard curve after measured standard protein samples. Then the concentration of the protein sample is report when the sample is measured.

**UV-VIS:** This type is designed for performing UV-VIS full spectrum( $200 \sim 850$ nm) absorbance scan with two path lengths : 1mm and 0.2mm.

#### • 1mm • 0.2mm

**Cell Culture:** This type is designed for measuring the concentration of suspended cell cultures with one path length 1mm.

**Micro Array:** This is designed for measuring the concentration of nucleic acid (at 260nm) and the concentration of fluorescent dye (at its highest absorption

peak) at the same time. Software provided 9 florescent dyes.

#### 4.3 Graph display area



The graph display area displays the scan curve of absorbance as below:

The red curve is the scan curve of absorbance generated each time after measured a sample.

There are three vertical measurement lines with different color (pink, yellow and blue) in the graph of which the corresponding wave lengths are the values of Xa Pos, Xb Pos and Xc Pos in the data display area respectively, and the corresponding absorbances are the values of Xa Value, Xb Value and Xc Value respectively.

Moving the mouse pointer nearby the measurement lines, the mouse pointer will change into a double headed arrow. When press the left mouse button down and move the mouse pointer, the measurement line will move, too. The measurement lines will move to the corresponding place and the absorbances will also change when the values of Xa Pos, Xb Pos and Xc Pos are changed. An absorbance curve will appear in the graph display area after measuring a sample. Tow curves will appear when both of the wavelengths: 1mm and 0.2mm are chosen using UV-VIS full-spectrum to scan a sample.

#### 4.4 Data display area

This area displays the wave length, absorbance, concentration of sample and others as below:

Xa Pos:	Xb Pos:	Xc Pos:	260/280
230	260	280	
Xa Value:	Xb Value:	Xc Value:	ng/ul
0.0000	0.0000	0.0000	

The absorbances of X Value correspond to the values of X Pos and the vertical measurement lines in the graph respectively.

The ratio of absorbance at 260nm and 280nm (the bar of 260/280) is used to assess the purity of nucleic acid.

Sample concentration in  $ng/\mu L$  is shown in the  $ng/\mu L$  bar for nucleic acid and in mg/mL for protein when a sample is measured.

#### 4.5 Measurement function area

There are five buttons in the measurement function area: "measure", "Blank", "Save Graph", "Show Report" and "View History Data" as below:



The "Blank" button is used to measure and store the blank which must be measured before measuring the samples.

The "Measure" button is used to measure the samples after measured a blank, and it is inactive and can't be used before a blank measured.

The "Save Graphic" button is used to save the graph after measured a sample. The "Show Report" button is used to output the result data of the samples by excel.

"View History Data" enables the user to browse all the results measured before.

In addition, there are "Sample Name" bar and "Sample ID" bar under the "Sample &Test Type". The user can input a name in the "Sample Name" bar and a number in the "Sample ID" bar for the sample when measuring it.

#### **5 Measurement**

#### 5.1 Nucleic acid

The YSA-400 micro-spectrophotometer can measure the concentration and purity of nucleic acids because nucleic acids have the highest absorption peak at 260nm, and the concentration of nucleic acids can be calculated and reported by the software based on the Beer-Lambert law, and the ratio of absorbance at 260nm and 280nm and the ratio of absorbance at 260nm and 280nm can assess the purity of nucleic acids.

5.1.1 Sample size requirements

Recommended volume: 2.0µL

5.1.2 Measurement range

DS-DNA: 2~3700ng/µL

SS-DNA: 2~2400ng/µL

RNA: 2~3000ng/µL

Typical reproducibility (SD= ng/ $\mu$ L; CV= %):

Sample range 2 $\sim$ 100ng/µL: ±2ng/µL

Sample range >100 ng/µL: ±2%

5.1.3 Work interface

Open the YSA-400 software and the default interface is the nucleic acids measurement interface as below:



#### Measurement type

Click "Sample & Test Type" to pop out a drop-down list box including "DS-DNA (50)", "SS-DNA(33)" and "RNA(44)" for measuring double-stranded DNA, single-stranded DNA and RNA respectively.

DS-DNA(50)	-
DS-DNA(50)	
SS-DNA(33)	

#### Wavelength and absorbance

There are three vertical measurement lines with different color (pink, yellow and blue) in the graph of which the corresponding wave lengths are the values of Xa Pos (230), Xb Pos(260) and Xc Pos(280) in the data display area respectively, and the corresponding absorbances are the values of Xa Value, Xb Value and Xc Value respectively, i.e. the values of Xa Value, Xb Value and Xc Value are the absorbances at 230nm, 260nm, and 280nm respectively(path length: 10mm).

#### 260/280

The ratio of absorbance at 260nm and 280nm can assess the purity of nucleic acids. Generally the ratio is about 1.8 for relative pure DNA and about 2.0 for relative pure RNA. A lower ratio indicates that the DNA or RNA may be contaminated by other things.

#### 260/230

The ratio of absorbance at 260nm and 230nm can also assess the purity of nucleic acids. Generally the ratio is  $1.8 \sim 2.0$  for relative pure DNA and RNA. The DNA and RNA may be contaminated by other things if the ratio is lower than 1.8.

#### Concentration

The concentration value of nucleic acids will appear in the ng/ $\mu$ L bar after measured a sample each time, and the unit is ng/ $\mu$ L.

5.1.4 Measurement procedures

For detailed measurement procedures, please see (4.2)

(1) Click the "Sample & Test Type" to select the right measurement type from "DS-DNA (50)", "SS-DNA (33)" and "RNA (44)". For example, select "DS-DNA

(50) "for measuring the double-stranded DNA samples.

(2) At first, measure a blank using the solvent by dropping it onto the lower measurement pedestal and clicking the "Blank" button to measure and store the blank.

(3) Wipe the solvent from the measurement pedestals with clear absorbent paper.

(4) Then drop sample onto the lower measurement pedestal and click the "measure" button to measure the sample.

(5) The result (values and graph) of the sample will appear in the work interface in seconds and the unique absorption spectrogram of "DS-DNA" is displayed as below:



(6) Click the "Save Graphic" button after measured a sample if you want to save the graph.

(7) When all the double-stranded DNA samples measurement finished, click the "Show Report" button to output the results by Excel.

#### 5.2 Protein A280

The YSA-400 micro-spectrophotometer can measure the concentration of proteins with two methods: protein A280 and the protein assay kits. Because proteins have the highest absorption peak at 280nm, the concentration of proteins can be calculated and reported by the software based on the Beer-Lambert law.

5.2.1 Sample size requirements

Recommended volume: 2.0µL

5.2.2 Measurement range

BSA: $0.1 \sim 100 \text{mg/mL}$ Typical reproducibility (SD= mg/mL; CV= %):Sample range $0.1 \sim 10 \text{mg/mL}$ :\$2mg/mL\$Sample range>10mg/mL:\$2%

#### 5.2.3 Work interface

Click the "Protein" button in the measurement type select area and enter the protein measurement interface as below:



#### Measurement type

Click the "Sample & Test Type" to pop out a drop-down list box, where "A280 (1)", "BSA(6.7)", "IgG(13.7)" and "Lysozyme (26.4) are designed for measuring protein at 280nm.

Sample&Test Type	
A280(1)	-
A280(1)	
BSA(6.7)	
lgG(13.7)	
Lysozyme(26.4)	
Labels	
BCA	
Bradford	
Lowry	

"A280 (1)" is designed for measuring the protein solution which produce an absorbance at 280nm of 1.0A (path length: 10mm) at the concentration of 1 mg/ml.

"BSA(6.7)", "IgG(13.7)" and "Lysozyme(26.4)" are designed for measuring the bovine serum albumin (BSA), immunoglobulin G(IgG) and Lysozyme respectively.

#### Wavelength and absorbance

The blue vertical measurement line corresponds to the wave length value of Xc Pos(280) and the absorbance corresponds to the Xc Value, i.e. the value of Xc Value is the absorbance at 280nm (path length: 10mm).

#### Concentration

The concentration value of proteins will appear in the mg/mL bar after measuring a sample each time, and the unit is mg/mL.

#### 5.2.4 Measurement procedures

For detailed measurement procedures, please see (4.2).

(1) Click the "Sample & Test Type" to select the right measurement type from "A280 (1)", "BSA(6.7)", "IgG(13.7)" and "Lysozyme(26.4)". For example, select "BSA(6.7)" for measuring the bovine serum albumin samples.

(2) At first, measure a blank using the solvent by dropping it onto the lower measurement pedestal and clicking "Blank" button to measure and store the

blank.

(3) Wipe the solvent from the measurement pedestals with clear absorbent paper.

(4) Then drop sample onto the lower measurement pedestal and click the "measure" button to measure the sample.

(5) The result (values and graph) of the sample will appear in the work interface in seconds and the typical absorption spectrogram of BSA is displayed as below:



(6) Click the "Save Graphic" button after measured a sample if you want to save the graph.

(7) When all the BSA samples measurement finished, click the "Show Report" button to output the results by Excel.

#### 5.3 BCA, Bradford and Lowry

The YSA-400 micro-spectrophotometer can measure the concentration of proteins of which the concentration is relatively lower or not pure using protein assay kits methods. These methods include BCA, Bradford and Lowry. Please see the protein assay kits instructions of BCA, Bradford and Lowry for the experimental principle and preparing for standard proteins and samples.

These methods need measure the standard proteins and generate a standard curve before measuring the samples. Then the concentration will be calculates and reported when a samples is measured. In this method, the concentration of the sample must be in the concentration range of the standard proteins.

5.3.1 Sample size requirements

Recommended volume: 2.0µL

5.3.2 Measurement range

	BCA	regula	$\cdot$ 0.2 $\sim$	8.0mg/mL	
		mini	0.01 $\sim$	0.2mg/mL	
	Bradford	regular	0.1~ 8	.0mg/mL	
		mini	0.015 $\sim$	0.1mg/mL	
	Lowry		0.2 ~	4.0mg/mL	
Typical rep	oroducibility	(SD= m	g/mL; C∖	/= %):	
BCA	Sample	range	0.01~0.	2mg/mL:	±0.1mg/mL
	Sample	range	0.2~8.	0mg/mL:	±2mg/mL
Bradford	Sample r	ange C	.015~0.0	5mg/mL:	±0.004mg/mL
	Sample	range (	0.05~0.12	5mg/mL:	±5%
	Sample	range	0.1~0.	5mg/mL:	±0.025mg/mL
	Sample	range	0.5~8.	0mg/mL:	±5 %
Lowry	Sample	range	0.2~4.	.0mg/mL:	±2mg/mL

#### 5.3.1 Work interface

Click the "Protein" button in the measurement types select area and enter the protein measurement interface.

#### Measurement type

Click the "Sample & Test Type" to pop out a drop-down list box, where "BCA", "Bradford" and "Lowry" are designed for measuring protein with protein assay kits methods.

#### Standard curve

These methods need make standard curve before measuring samples. The "Sample" and "Standard" buttons will appear above "Sample & Test Type" When the user selects one of the BCA, Bradford and Lowry. Click the "Standard" button, a table will appear under it as below:

Name	Conc	Ave.Abs	^	

The data will be shown in the table when measuring a standard protein. The column of "Ave.Abs" shows the absorbances of standard proteins. And the user can input the concentration of the standard protein in the column of "Conc".

A work curve will form by clicking the right mouse button in the standard table and selecting the "Work Curve" after measured all the standard proteins. "Delete All Item" enables the user to delete all the data. "Delete Item" can delete one row. "Load Standard File" can load the data of a standard file saved in the computer. "Save Standard File" can save the data of the standard protein.

Name	Conc	Ave.	Abs
Γ	Delete All	Item	Ctrl+D
	D <u>e</u> lete Ite	m	Ctrl+I
	<u>W</u> ork Curve Load Standard Fil		Ctrl+W Ctrl+L
	Save Stand	ard File	Ctrl+S

#### Wavelength and absorbance

The vertical measurement line corresponds to the wavelength of 560nm for measuring the protein with BCA method, 595nm for Bradford and 660nm for Lowry.

#### Concentration

The concentration value of proteins will appear in the mg/mL bar after measuring a sample each time, and the unit is mg/mL.

#### 5.3.4 Measurement procedures

For detailed measurement procedures, please see (4.2).

(1) Click the "Sample & Test Type" to select the right measurement type from "BCA", "Bradford" and "Lowry". For example, select "BCA" for measuring the proteins with BCA protein assay kit.



(2) Click "Standard" to measure the standard proteins.

(3) At first, measure a blank.

(4) Wipe the solvent from the measurement pedestals.

(5) Measure at least five standard proteins with different concentrations.

(6) Click the right mouse button in the standard table and select the "Work Curve" to generate a standard curve.

(7) Then click "Sample" to measure the samples.

(8) Measure samples and the result (values and graph) will appear in the work interface in seconds.

(8) Click the "Save Graphic" button after measured a sample if you want to store the graph.

(9) Click the "Show Report" button after the samples measured to output the result by Excel.

#### **5.4 UV-VIS**

The YSA-400 micro-spectrophotometer can make UV-VIS full spectrum(200 $\sim$ 850nm) absorbance scan.

5.4.1 Sample size requirements

Recommended volume: 2.0µL

5.4.2 Measurement range

	0.1Abs $\sim$ 75Abs	
Typical reproducibility	(SD=Abs; CV=%)	
Sample range	0.1Abs $\sim$ 5Abs:	±0.1
Sample range	5 $\sim$ 75Abs:	±2 %

#### 5.4.3 Work interface

Click the "UV-VIS" button in the measurement type select area and enter the UV-VIS full spectrum absorbance scan interface.

#### Wavelength and absorbance

There are three vertical measurement lines with different color (purple, yellow and blue) in the graph of which the corresponding wave lengths are the values of Xa Pos, Xb Pos and Xc Pos in the data display area respectively, and the corresponding absorbances are the values of Xa Value, Xb Value and Xc Value respectively. User can move the measurement lines to the appropriate place based on the sample.

#### 5.4.4 Measurement procedures

For detailed measurement procedures, please see (4.2).

(1) At first, measure a blank using the solvent by dropping it onto the lower measurement pedestal and clicking "Blank" button to measure and store the blank.

(2) Wipe the solvent from the measurement pedestals with clear absorbent paper.

(3) Then measure the samples.

(4) The result (values and graph) of the sample will appear in the work interface in seconds and the typical absorption spectrogram of  $\kappa_2 Cr_2 O_7$  is displayed as below:



(5) Click the "Save Graphic" button after measured a sample if you want to store the graph.

(6) Click the "Show Report" button after the samples measured to output the result by Excel.

#### 5.5 Cell Cultures

The YSA-400 micro-spectrophotometer can measure the concentration of suspended cell cultures using one path length: 1mm.

5.5.1 Sample size requirements

Recommended volume: 2.0µL

5.5.2 Measurement range

	0.1Abs $\sim$ 75Abs	
Typical reproducibility	(SD=Abs; CV=%)	
Sample range	0.1Abs $\sim$ 5Abs:	±0.1
Sample range	5 $\sim$ 75Abs:	±2 %

#### 5.5.3 Work interface

Click the "Cell Cultures" button in the measurement type select area and enter the cell cultures measurement interface.

#### Wavelength and absorbance

The blue vertical measurement line corresponds to the wave length value of Xc Pos(600) and the absorbance corresponds to the Xc Value, i.e. the value of Xc Value is the absorbance at 600nm (path length: 10mm). The user can move the vertical measurement line to the appropriate place to get the corresponding absorbance.

#### Path length

The software provided one path length 1mm for measuring cell cultures. A scan curve will appear in the graph display area when measuring a sample.

#### 5.5.4 Measurement procedures

For detailed measurement procedures, please see (4.2).

(1) At first, measure a blank using the culture solution without cells by dropping it onto the lower measurement pedestal and clicking "Blank" button to measure and store the blank. (2) Wipe the solution from the measurement pedestals with clear absorbent paper.

(3) Then measure the samples.

(4) The result (values and graph) of the sample will appear in the work interface in seconds and the typical absorption spectrogram of BCA is displayed.

(5) Click the "Save Graphic" button after measured a sample if you want to store the graph.

(6) Click the "Show Report" button after the samples measured to output the result by Excel.

#### 5.6 Micro Array

The YSA-400 micro-spectrophotometer can measure the concentration of fluorescent dye labeled nucleic acid (micro array), for it can measure the concentration of the nucleic acid and the dyes at the same time. The concentration of nucleic acids and the fluorescent dyes can be calculated and reported by the software based on the Beer-Lambert law. The software provided 10 fluorescent dyes for you to select.

5.6.1 Sample size requirements

Recommended volume: 2.0µL

5.4.2 Measurement range

DNA: 2~750ng/µL Typical reproducibility (SD=  $ng/\mu L$ ; CV= %): Sample range 2~100ng/µL: ±2ng/µL Sample range >100ng/µL: ±2% Cy3: 0.2~100pmol/µL Typical reproducibility (SD= ng/ $\mu$ L; CV= %): Sample range  $0.2 \sim 4.0 \text{pmol/}\mu\text{L}$ : ±0.2pmol/µL >4.0pmol/µL: **±2**% Sample range

5.6.3 Work interface

Click the "Micro Array" button in the measurement type select area to enter the micro array measurement interface as below:



#### Measurement type

**Nucleic Acids:** Click "Sample & Test Type" to pop out a drop-down list box including "DS-DNA", "SS-DNA" and "RNA" for measuring double-stranded DNA, single-stranded DNA and RNA respectively at 260nm.

**Fluorescent dyes:** 10 fluorescent dyes are provided by the software. The respective extinction coefficient, absorbance wavelength and 260nm and 280nm % corrections are shown in the table by clicking the "Dye Editer" submenu under the "Tool" main menu.

Name	1/M-cm	nm	260nm%	280nm%	Save & Exi
СуЗ	150000.000000	550	0.040000	0.050000	002120
Cy5	250000.000000	650	0.000000	0.050000	Exit
Alexa Fluor 488	710000.000000	495	0.300000	0.110000	
Alexa Fluor 546	104000.000000	556	0.210000	0.120000	
Alexa Fluor 555	150000.000000	555	0.040000	0.080000	
Alexa Fluor 594	73000.000000	590	0.430000	0.560000	
Alexa Fluor 647	239000.000000	650	0.000000	0.030000	
Alexa Fluor 660	132000.000000	663	0.000000	0.100000	
Cy3.5	150000.000000	581	0.080000	0.240000	
Cy5.5	250000.000000	675	0.050000	0.180000	
		-			

The user can also select the dyes from the drop-down list box. The default setting is that the Dye1 is Cy3 and the Dye2 is Cy5.

#### Wavelength and absorbance

The pink vertical measurement line corresponds to the wavelength of 260nm for measuring the nucleic acids and the corresponding absorbance are shown in the Xa Value.

The yellow and blue vertical measurement lines correspond to the wavelengths of the dyes for measuring the dyes and the corresponding absorbance are shown in the Dye1 Abs and Dye2 Abs respectively.

#### Concentration

The value of nucleic concentration will appear in ng/ $\mu$ L bar after measuring a sample each time, and the unit is ng/ $\mu$ L. The value of dye concentration will appear in the  $\mu$ M bar and the unit is pmol/ $\mu$ L.

#### 5.6.4 Measurement procedures

For detailed measurement procedures, please see (4.2).

(1) Click the "Micro Array" to enter the micro array measurement interface.

(2) Click the "Sample & Test Type" and select the right measurement type of nucleic acid from "DS-DNA (50)", "SS-DNA (33)" and "RNA (44)". For example, select "SS-DNA(33)" for measuring the single-stranded DNA and dye samples.
(3) Click the "dyes" button to select the right measurement type of dye from 10dyes, For example, choose "Cy3" for measuring the SS-DNA and Cy3 samples, and/or choose "Cy5" for measuring the SS-DNA and Cy3 and/or Cy5 samples.

(4) At first, measure a blank using the solvent by dropping it onto the lower measurement pedestal and clicking "Blank" button to measure and store the blank.

(5) Wipe the solvent from the measurement pedestals with clear absorbent paper.

(6) Then drop sample onto the lower measurement pedestal and click the "measure" button to measure the sample.

(7) The result (values and graph) of the sample will appear in the work interface in seconds.

(8) Click the "Save Graphic" button after measured a sample if you want to store the graph.

(9) Click the "Show Report" button after the samples measured to output the result by Excel.