Microdrop2000

UV-VIS SPECTROPHOTOMETER

User Manual

VER 1.2

Copyright Statement

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Sample Usage Reminder

Place the Microdrop2000 in a location **free from direct airflow or exhaust fans** to minimize evaporation and ensure measurement accuracy.

The **Microdrop2000** is a high-precision, high-reproducibility full-spectrum UV-Vis spectrophotometer featuring a built-in touchscreen and pre-installed software. Operable over a wavelength range of **190–850 nm**, it requires only **~2 µL** of sample volume. The instrument is user-friendly, easy to clean, and built for routine and advanced laboratory use

This microvolume spectrophotometer accurately quantifies the concentration and purity of **double-stranded DNA, single-stranded DNA, and RNA**. It is also perfectly suited for **protein concentration assays**, **OD_{600} cell and bacterial density measurements**, as well as full-wavelength UV-Vis scanning and other spectroscopy applications.

1.1 Functions

The **Microdrop2000** is equipped with both a **microvolume sample detection system** and a **cuvette detection system**, allowing for the analysis of diluted samples using standard UV- Vis cuvettes.

1.1.1Touchscreen

The Microdrop2000 features a built-in **7-inch high-definition touchscreen** with pre-installed, user-friendly instrument control software. The display is designed with a **10° tilt angle**, making it convenient for both standing and seated operation, while ensuring optimal visual performance.

1.1.2 Cuvette Holder

The Microdrop2000 includes a cuvette holder for analyzing diluted samples, performing colorimetric analyses, conducting cell culture measurements, and supporting kinetic studies.

1.1.3 USB Port

A USB port located on the side of the instrument allows for **data export** or **software upgrades**.

1.2 Terms and Definitions

1.2.1 A230, A260, A280

The absorbance values measured at **230 nm, 260 nm, and 280 nm**, respectively. These values are normalized to a 10 mm optical path length, equivalent to 10× the absorbance measured at a 1 mm path length, or 50× the absorbance at a 0.2 mm path length.

1.2.2 A260/A280

The ratio of absorbance at 260 nm and 280 nm, commonly used to evaluate **DNA or RNA purity**:

Pure DNA typically yields a ratio of **~1.8** (range: 1.7–1.9).

Pure RNA typically yields a ratio of **~2.0** (range: 1.9–2.1).

Ratios >1.9 for DNA may indicate RNA contamination.

Ratios <1.7 for DNA or <1.9 for RNA suggest contamination by phenol or protein.

Ratios >2.1 for RNA may indicate residual guanidine isothiocyanate.

1.2.3 A260/A230

The ratio of absorbance at 260 nm and 230 nm. This is a **secondary indicator of nucleic acid purity**. Pure nucleic acids usually have ratios **≥2.0**. Values below 2.0 may indicate contamination by carbohydrates, salts, or organic solvents, requiring sample purification.

1.2.4 Sample Concentration

The concentration of the tested sample. Units are **ng/μL for DNA and RNA**, and **mg/mL for proteins**.

Concentration Calculation Formulas:

Sample Type	Item	Formula
Nucleic Acids	dsDNA	C = A260 × 50

Sample Type (ng/μL)	Item	Formula
	ssDNA	C = A260 × 33
	RNA	C = A260 × 40
	Custom	C = A260 × user-defined conversion factor
Proteins (mg/mL)	General	C = A280
	BSA	C = A280 × 10 ÷ 6.7
	IgA	C = A280 × 10 ÷ 13.7
	Lysozyme	C = A280 × 10 ÷ 26.4
	Molar Extinction Coefficient Method	C = A280 × MW ÷ εmolar
	Percent Extinction Coefficient Method	C = A280 ÷ εpercent × 10
	Custom	C = Absorbance × user-defined factor

1.3 Accessories

The Microdrop2000 is supplied with a power adapter (**AC 110–220 V, 1.8 A, 50–60 Hz; output: DC 24 V, 1 A**).

1.4 Instrument Application Scope

The instrument is suitable for:

Concentration and purity testing of **dsDNA, ssDNA, and RNA**.

Protein quantification using multiple methods.

Measurement of **cell and bacterial concentrations**.

Routine **UV- Vis full wavelength scanning** and other spectrophotometric applications.

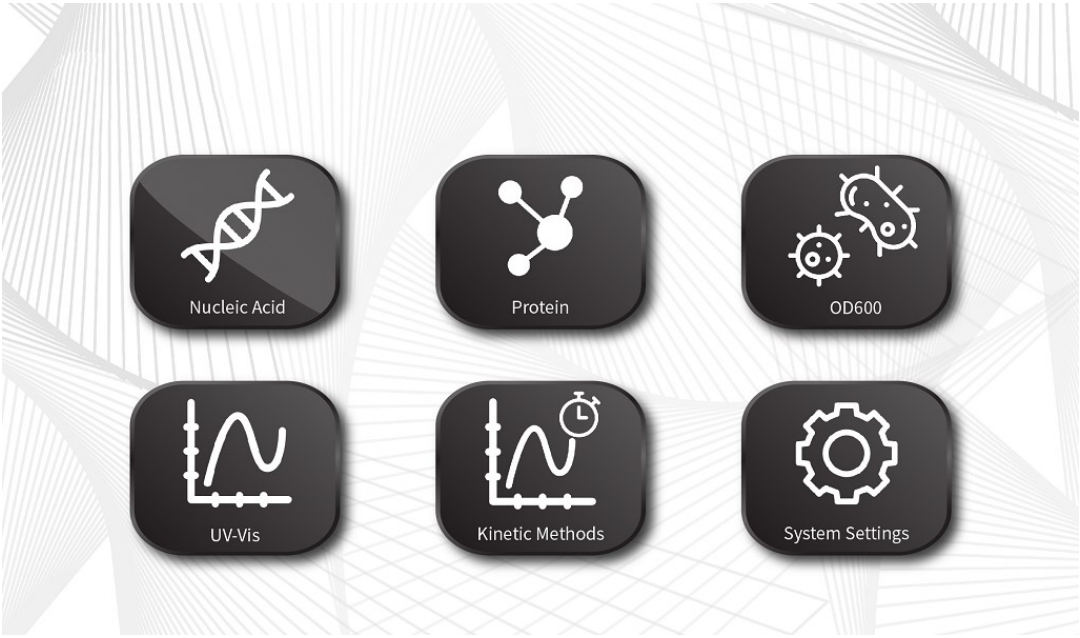
1.5 Detection Range

Detection Position	Pathlength (mm)	Upper Limit (10 mm Equivalent Absorbance)
Pedestal	1.0	12.5
	0.2	62.5
	0.02	750
Cuvette	10.0	1.5

2. Pre-Use Features

2.1 Power On

Connect the data cable and power adapter. Switch on the instrument. After approximately **30 seconds**, the main interface will appear.



2.2 Software Installation or Update

To update the Microdrop2000 software:

Copy the new software file from a computer to a USB drive. Do not rename the file.

Insert the USB drive into the Microdrop2000's USB port.

On the **Home** screen, select **Settings → Update Software**, and wait for completion.

Restart the instrument.

2.3 Data Saving and Export

Screenshot: Tap the "screenshot" button (top-left grid icon) to save the current interface. Images are saved automatically to the "screenshot" folder on the device.

Export: Tap the "Export" button (bottom of the screen) to save data in Excel format to a FAT32-formatted USB drive.

Save: Tap the "Save" button to store data on the internal storage card in the "date" folder.

.CSV: Table data only

_Spec.csv: Absorbance data across wavelengths

.date: Full dataset including absorbance and tables

Data saved on the device can also be exported via **System Settings → Export** to a USB drive (FAT32). Files are named “ums”.

2.4 Keyboard and Mouse Functionality

While the default control is via touchscreen, users may connect a keyboard or mouse:

Go to **System Settings → System Information → Other**.

Select **Show Cursor**.

Insert keyboard/mouse into the USB port. For both, use a USB hub.

2.5 Sample Volume (Recommended)

Pedestal Measurements:

Nucleic acids: 1–2 μL

Proteins: 2 μL

Cell suspensions: 2 μL

Other samples: 2 μL

Note: A sufficient liquid column must form between the pedestal surfaces for accurate measurement.

Low surface tension samples may require ~2 μL for stability.

For accuracy, use a high-precision micropipette (0–2.5 μL). Lower precision pipettes (0–10 μL) may not ensure reliable transfer.

Cuvette Measurements:

For standard 10 mm cuvettes, use at least 2 mL of sample, or follow manufacturer recommendations. Ensure the light path passes through the sample.

2.6 Application Detection Range

Default units: ng/μL for nucleic acids, mg/mL for proteins.

The table below provides approximate detection limits for microvolume analysis (pedestal).

For 10 mm cuvettes, absorbance range is **0–1.5 A**.

Sample Type	Lower Limit	Upper Limit	Reproducibility

Sample Type	Lower Limit	Upper Limit	Reproducibility
dsDNA	2.0 ng/µL (pedestal); 0.20 ng/µL (cuvette)	37,500 ng/µL (pedestal); 75 ng/µL (cuvette)	±2.0 ng/µL (2–100 ng/µL); ±2% (>100 ng/µL)
ssDNA	2.0 ng/µL (pedestal); 0.20 ng/µL (cuvette)	24,750 ng/µL (pedestal); 75 ng/µL (cuvette)	±2.0 ng/µL (2–100 ng/µL); ±2% (>100 ng/µL)
RNA	2.0 ng/µL (pedestal); 0.20 ng/µL (cuvette)	30,000 ng/µL (pedestal); 75 ng/µL (cuvette)	±2.0 ng/µL (2–100 ng/µL); ±2% (>100 ng/µL)
A280 Proteins	0.06 mg/mL (pedestal); 0.20 mg/mL (cuvette)	750 mg/mL (pedestal)	±0.10 mg/mL (0.10–10 mg/mL); ±2% (>10 mg/mL)
BSA	0.06 mg/mL (pedestal); 0.006 mg/mL (cuvette)	1100 mg/mL (pedestal)	±0.10 mg/mL (0.10–10 mg/mL); ±2% (>10 mg/mL)
Protein BCA	0.20 mg/mL (20:1 reagent:sample); 0.01 mg/mL (1:1)	8.0 mg/mL (pedestal); 0.20 mg/mL (cuvette)	±2% full range; ±0.01 mg/mL (low conc.)
Protein Bradford	100 µg/mL (50:1 reagent:sample); 15 µg/mL (1:1)	8000 µg/mL (pedestal); 100 µg/mL (cuvette)	±25 µg/mL (100–500 µg/mL); ±5% (500–8000 µg/mL); ±4 µg/mL (15–50 µg/mL)
Protein Lowry	0.20 mg/mL (pedestal)	4.0 mg/mL (pedestal)	±2% full range

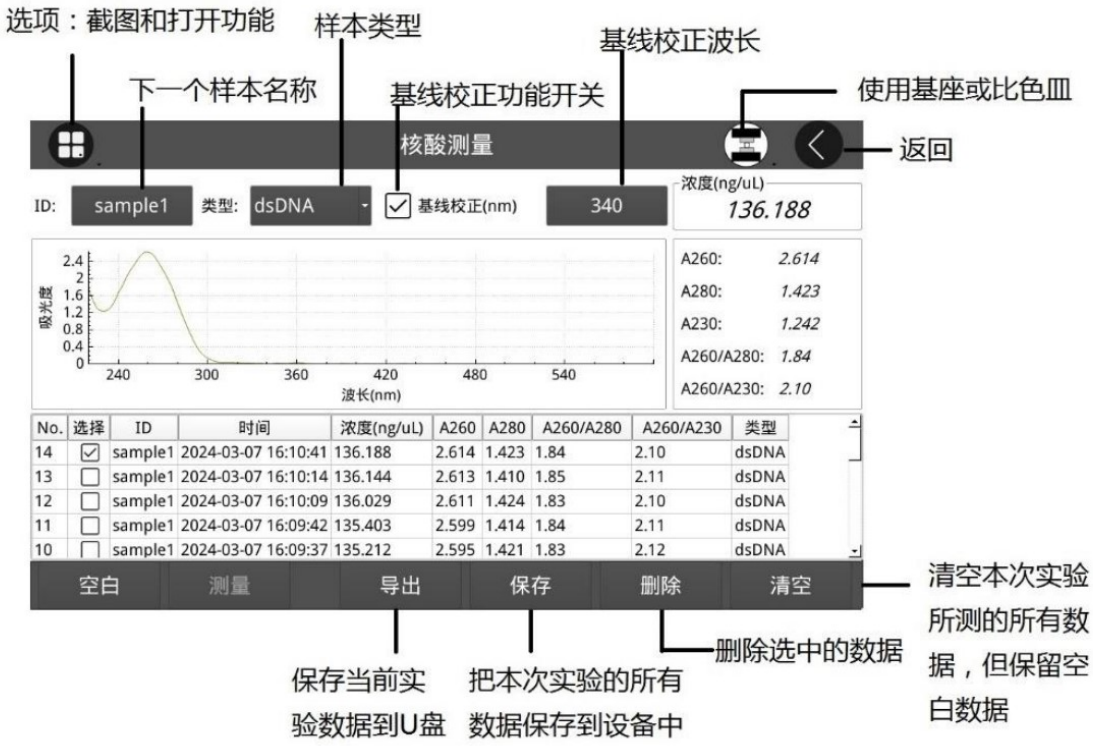
3. Nucleic Acid Measurement

3.1 Measurement of Double-Stranded DNA, Single-Stranded DNA, or RNA

The absorbance at 260 nm is measured, along with the ratios of two absorbance values (A260/A280 and A260/A230), and the sample concentration.

Cautions

Do not use pipettors or spray bottles near the instrument, as liquid may enter the device and cause permanent damage.



Do not use hydrofluoric acid (HF) on the pedestal. Fluoride ions can permanently damage quartz fiber optics.

Preparation Before Testing

Before performing pedestal measurements with the Microdrop2000, lift the measurement arm and clean both the upper and lower pedestals. At minimum, wipe the pedestal with a new laboratory-grade lint-free tissue.

Alternatively, lift the arm, apply 2 μL of blank solution or ultrapure water onto the pedestal, lower the arm, then raise it again and wipe both pedestals clean with lint-free tissue.

Measurement Procedure

On the Home screen, tap **“Nucleic Acid Measurement”** to enter the nucleic acid measurement interface. Default baseline calibration is at 340 nm.

Pipette 1–2 μL of blank solution onto the lower pedestal, then lower the arm.

Or, insert a blank cuvette into the holder and close the lid.

Note: If using a cuvette, align its optical path with the instrument’s beam path.

Tap **“Blank”** and wait for measurement to complete.

Raise the arm and wipe both pedestals with fresh lint-free tissue, or remove the blank cuvette.

Pipette 1–2 μL of the sample solution onto the pedestal, then lower the arm.

Or, insert the sample cuvette and close the lid.

Start the sample measurement:

Pedestal: Lower the arm and tap **“Measure”**.

Cuvette: Tap **“Measure”**.

After measurement, raise the arm and wipe both pedestals, or remove the sample cuvette. Continue with new samples as needed.

When finished, wipe the pedestal clean and either:

Tap the upper-right **Return** button to go back to the Home screen,

Tap **“Export”** to export results, or

Tap **“Save”** to store the current experiment’s data.

To delete results: Select up to 5 rows in the “Select” column and tap **“Delete”**.

 Recommendation: While it is not necessary to blank before every measurement, we recommend performing a blank calibration every 30 minutes when measuring multiple samples.

3.2 Measurement of Oligonucleotide DNA and Oligonucleotide RNA

For oligonucleotide quantification, absorbance at 260 nm is measured. The molar extinction coefficient is automatically calculated based on the user-defined nucleotide sequence. Reports include nucleic acid concentration and the absorbance ratios (A260/A280 and A260/A230). Single-point baseline correction may also be applied. Oligonucleotide calculation principles differ from general nucleic acids (see Appendix).

Preparation Before Testing

Same as Section 3.1.

Measurement Procedure

On the Home screen, tap **“Nucleic Acid Measurement”** and select **Oligo DNA** or **Oligo RNA** based on sample type.

Tap the **Oligonucleotide** button and input the nucleotide sequence. Return to the measurement interface. Default baseline calibration is at 340 nm.

Pipette 1–2 μL of blank solution onto the pedestal, then lower the arm, or insert a blank cuvette.

Note: Ensure the cuvette optical path is aligned.

Tap **“Blank”** and wait for completion.

Clean pedestal or remove cuvette as described above.

Pipette 1–2 μL of sample solution, then lower the arm, or insert the sample cuvette.

Tap **“Measure”** to perform the measurement.

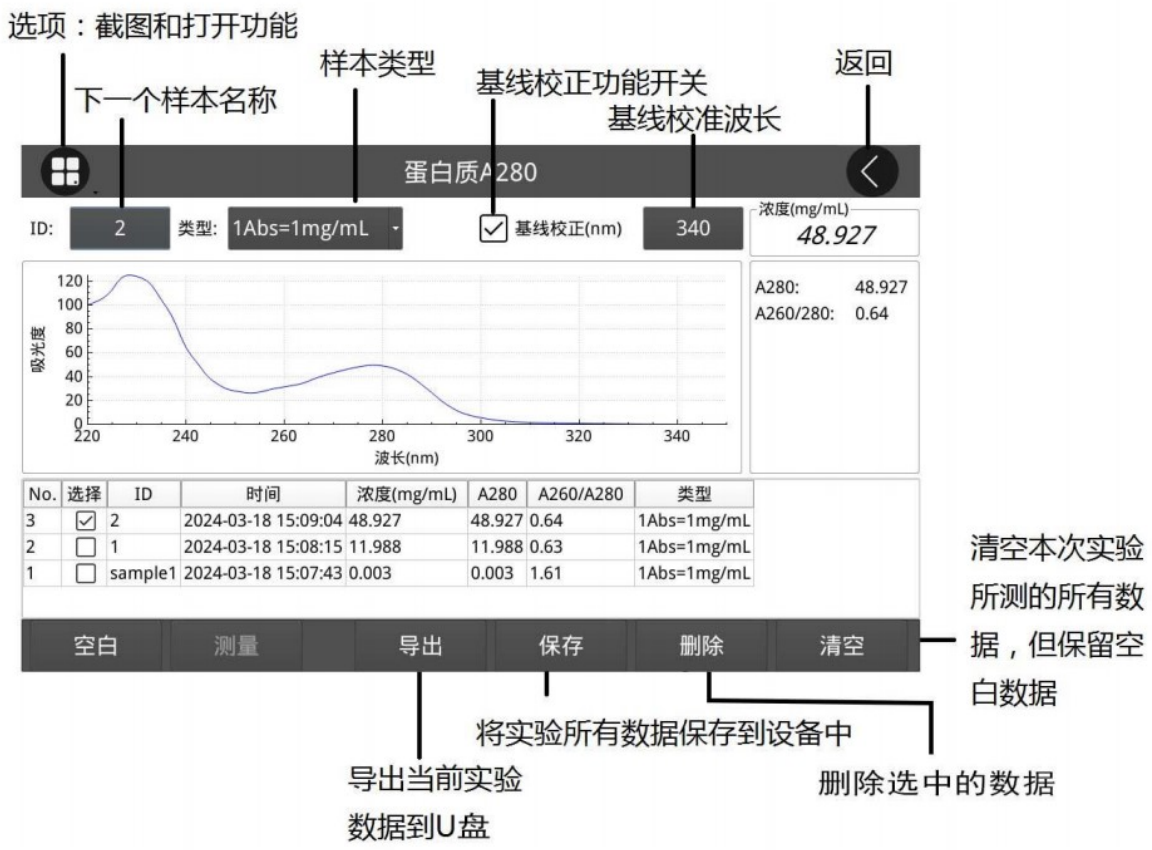
Clean pedestal or remove cuvette after each sample.

Export or save results as needed.

 Recommendation: Perform blank calibration every 30 minutes when testing multiple samples.

1 Protein Measurement

4.1 A280 method



Preparation Before Testing

Before starting pedestal measurements with the Microdrop2000 instrument, lift the detection arm and clean both the upper and lower pedestals using new lint-free laboratory wipes.

Alternatively, lift the detection arm, place 2 μL of blank solution or purified water on the pedestal, lower and raise the detection arm, then wipe both pedestals with lint-free wipes.

Procedure

On the home screen, click the **Protein Detection** button and select **Protein A280** to enter the protein A280 measurement interface.

Choose the appropriate mode according to the sample type:

1Abs = 1 mg/mL

BSA

IgA

Lysozyme

Other Protein

ε + MW

ε 1%

In the *Other Protein* mode, the correction factor defaults to **1** and can be adjusted according to the sample characteristics.

For the **ε+MW** and **ε 1%** modes, click the *Extinction Coefficient* button in the upper right corner. Enter the required information based on known parameters, then return to the measurement interface. Baseline calibration is enabled by default, using a reference wavelength of **340 nm**.

Molar Extinction Coefficient Method (ε+MW)

When using this method, two key pieces of information are required:

The **molar extinction coefficient (εmolar)** of the target protein, *or* the number of tryptophan (Trp, W), tyrosine (Tyr, Y), and cysteine (Cys, C) residues.

The molar extinction coefficient at 280 nm for a peptide or protein is determined by its content of Trp, Tyr, and Cys residues.

For more details, see Appendix F: *Protein Extinction Coefficients*.

The **molecular weight (MW)** of the target protein.

Procedure:

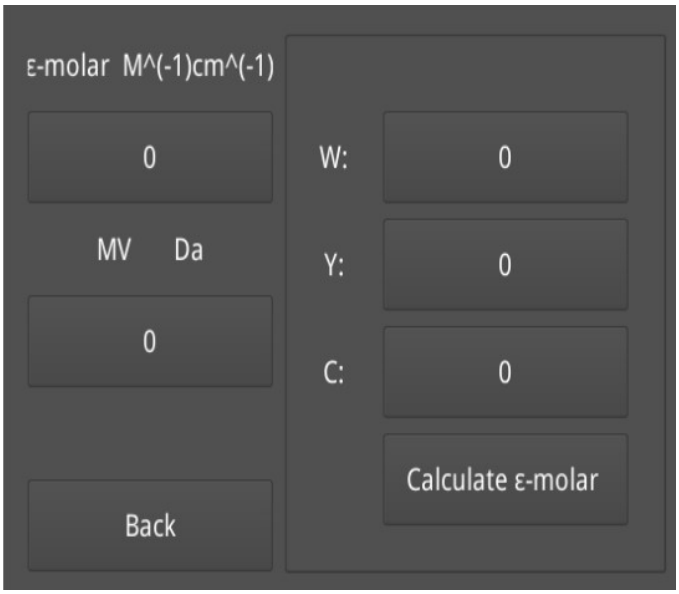
a. From the protein type dropdown menu on the right panel, select **ε+MW (Molar Extinction Coefficient Method)**.

b. In the pop-up window *Set Molar Extinction Coefficient and Molecular Weight*, enter the known εmolar in the designated field.

If εmolar is unknown, use the built-in calculator on the right: input the number of Trp, Tyr, and Cys residues, then click **Calculate** to obtain the molar extinction coefficient.

c. Enter the molecular weight of the target protein in the MW field.

d. Click **Return** to go back to the measurement interface.



Percent Extinction Coefficient Method (ε 1%)

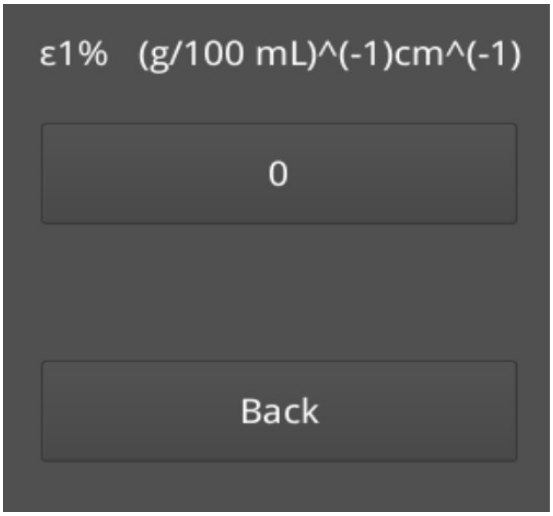
a. From the protein type dropdown menu on the right panel, select **ε 1% (Percent Extinction Coefficient Method)**.

b. In the pop-up window *Set Percent Extinction Coefficient*, enter the value for **ε 1%**.

ε 1% refers to the absorbance at 280 nm of a 1% (g/100 mL) protein solution measured in a 1 cm path length cuvette.

If the known absorbance value is for a 0.1% (mg/mL) protein solution, multiply that value by 10 before entering it.

c. Click **Return** to go back to the measurement interface.



Measurement Procedure

1.

Pipette **1–2 µL of blank solution** onto the lower pedestal, then lower the detection arm, or insert the blank cuvette into the cuvette holder.

2.

Tip: If using a cuvette, ensure the cuvette's optical path is aligned with the instrument's light path.

3.

4.

Click **Blank** and wait for the measurement to complete.

5.

6.

Raise the detection arm and wipe both the upper and lower pedestals with a new piece of lint-free lab wipe, or remove the blank cuvette.

7.

8.

Pipette **1–2 µL of the sample solution** onto the pedestal, then lower the detection arm, or insert the sample cuvette into the cuvette holder and close the lid.

9.

10.

Start sample measurement:

11.

1.

Pedestal: Lower the detection arm and click **Measure**.

2.

3.

Cuvette: Click **Measure**.

4.

12.

Raise the detection arm and wipe both the upper and lower pedestals with a new lint-free wipe, or remove the sample cuvette. Add the next sample and repeat as needed.

13.

14.

After completing the experiment, raise the detection arm and clean the pedestals again with a new lint-free wipe, or remove the final sample cuvette.

15.

1.

Click the top-right button to **Return to Main Menu**, or click **Export** to export the current experiment data, or click **Save** to save the data.

2.

Note: Although it is not necessary to perform a blank measurement before every sample, we recommend performing a blank calibration at least once every **30 minutes** when testing multiple samples.

4.2 Protein Quantification Methods: BCA, Bradford, Lowry, Pierce 660, and Others

These detection methods are generally similar and are described together.

Principle Overview

BCA Assay

The Bicinchoninic Acid (BCA) assay uses bicinchoninic acid as a chromogenic reagent to detect Cu^{+} ions. Certain proteins reduce Cu^{2+} to Cu^{+} under alkaline conditions. The resulting purple complex is formed when two BCA molecules chelate one Cu^{+} ion. The Cu-BCA complex is detected at **562 nm**, with baseline correction at **750 nm**.

Bradford Assay

The Bradford assay uses Coomassie Brilliant Blue dye to estimate the total protein concentration in unpurified protein samples. It is particularly useful for diluted protein solutions or proteins that show significant absorbance between **200 nm and 280 nm**, where

direct measurement at 280 nm or 205 nm is unsuitable. The protein-dye complex is detected at **595 nm**, with baseline correction at **750 nm**. Quantification is performed using a standard curve.

Lowry Assay

In the Lowry method, proteins react with copper sulfate in alkaline solution, leading to the formation of a mixture of phosphotungstic and phosphomolybdic blues. The Folin–Ciocalteu reagent further reduces the copper-protein complex. The water-soluble blue product is detected at **650 nm**, with baseline correction at **405 nm**.

Pierce 660 Assay

This assay is based on a proprietary dye–metal complex that binds proteins under acidic conditions, shifting the dye's maximum absorbance to **660 nm**. The dye–metal complex is red-brown, but turns green upon binding protein due to deprotonation from interactions with positively charged amino acid residues (e.g., histidine, arginine, lysine), and to a lesser extent tyrosine, tryptophan, and phenylalanine. Baseline correction is performed at **750 nm**. The resulting color is stable and increases proportionally with protein concentration. An optional **Ionic Detergent Compatibility Reagent (IDCR)** may be added to improve assay compatibility with high concentrations of ionic detergents, such as those in Laemmli SDS sample buffer with bromophenol blue. After mixing, IDCR fully dissolves and does not interfere with measurement.

Preparation Before Testing

Before using the **Microdrop2000 instrument** for pedestal measurements, raise the detection arm and clean both the upper and lower pedestals. Use a new piece of lint-free laboratory tissue at minimum.

Alternatively, raise the detection arm, pipette **2 µL of blank solution or pure water** onto the pedestal, lower and then lift the detection arm, and wipe the pedestals with lint-free tissue.

Detection Methods and Parameters

Detection Method	Detection Wavelength (nm)	Baseline Wavelength (nm)	Recommended Curve Type	Blank Control
Protein A280 Method	280	340	None	Reference solution
Protein BCA Method	562	750	Linear	Deionized water
Protein Bradford Method	595	750	Quadratic polynomial	Deionized water
Protein Lowry Method	650	405	Quadratic polynomial	Deionized water
Protein Pierce 660 Method	660	750	Linear	Reference solution
Protein Others Method	Custom	Custom	Custom	Custom

Standard Workflow

Enter Detection Mode

On the main interface, click **Protein Detection**, then select the desired detection method to enter the corresponding measurement interface.

Blank Measurement

Click the **Standard Curve** tab on the left to enter the standard curve interface.

Select the curve type as recommended in the table above.

Pipette **2 µL of blank solution** (reference solvent or deionized water, depending on the experiment) onto the lower pedestal, then lower the detection arm, or insert a blank cuvette into the holder.

Click **Blank** and wait for measurement to complete.

Tip: If using a cuvette, ensure the optical path aligns with the instrument light path.

Raise the detection arm and wipe both pedestals with new lint-free tissue, or remove the blank cuvette.

Measure Reference Standard Sample

Pipette **2 μL of reference standard solution** onto the pedestal, then lower the detection arm, or insert the standard cuvette and close the lid.

Select the **standard ID (std-1)**, enter the corresponding concentration in the input box, and click **Measure**.

If needed, repeat the measurement.

Measure Remaining Standards

Pipette **2 μL of standard solution** (e.g., standard 1) onto the pedestal, then lower the detection arm, or insert the cuvette and close the lid.

Select the corresponding **standard ID (std-2, std-3, etc.)**, enter the concentration, and click **Measure**.

Repeat as needed for standards 2, 3, 4, etc.

To delete a data entry, click anywhere on the corresponding row in the table. A prompt will appear asking **"Delete this entry?"** Choose as required.

After completing all standard measurements, click **Create Standard Curve** to generate the curve according to the selected fitting method.

Measure Samples

(Procedure continues with sample measurement as per standard curve.)

1.

Click the **Sample Detection** tab on the left to enter the sample detection interface.

Pipette **2 μL of Sample 1 solution** onto the lower pedestal, then lower the detection arm, or insert the **Sample 1 cuvette** into the cuvette holder and close the lid.

Click **"Measure"**. If necessary, repeat the measurement.

Remove the sample cuvette, then add the next sample and proceed in sequence.

After the experiment is completed, lift the detection arm and wipe the upper and lower pedestals with a new piece of lint-free laboratory tissue, or remove the sample cuvette.

Click the **Return to Main Interface** button in the upper right corner, or click **"Export"** to export the current experimental data, or click **"Save"** to save the data from this experiment.

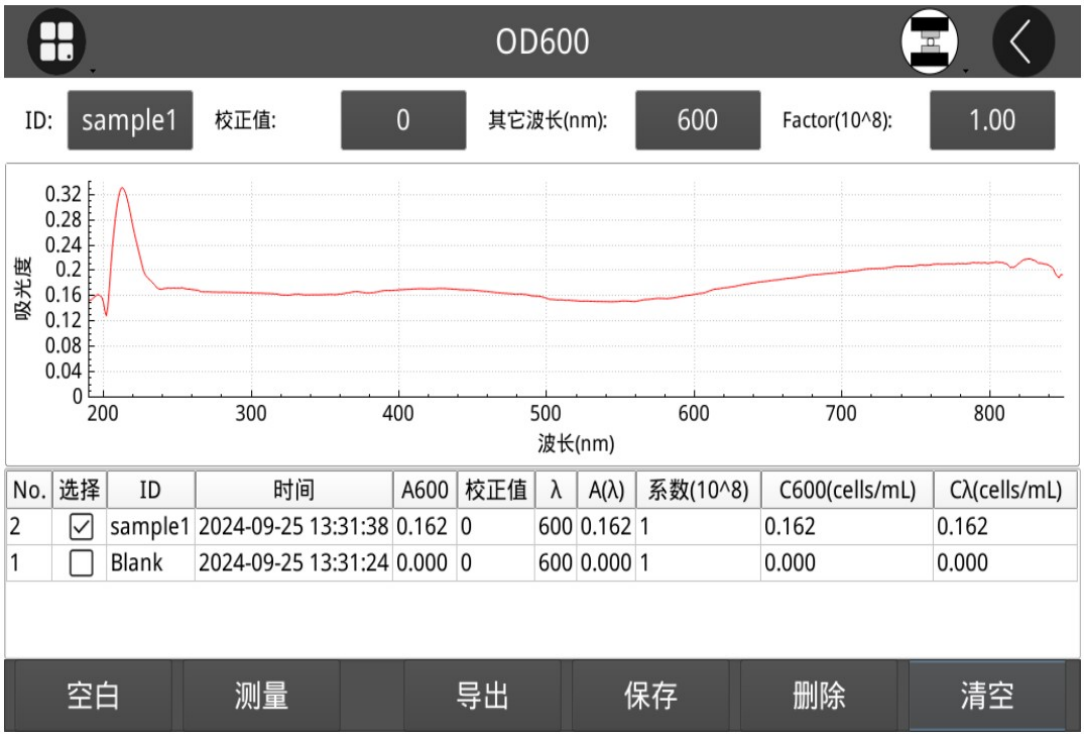
Note: Although it is not necessary to perform a blank test before each sample, we recommend performing a blank calibration every 30 minutes when testing multiple samples.

2 OD600 Measurement

By measuring the optical density (absorbance) of cell cultures at 600 nm, the OD600 application monitors the growth rate of bacteria or other microbial cell cultures. The Beer–Lambert equation, combined with a user-defined conversion factor, is used to correlate absorbance with concentration. The reported concentration values can be used to determine the growth stage of the cultured cell population, such as logarithmic, exponential, or stationary phase.

The OD600 application reports cell concentration in units of cells/mL. Single-point absorbance correction can be applied. This application does not require a standard curve.

Note: Due to the presence of a certain amount of scattered light in this analysis, the absorbance readings are typically very low.



OD600 Measurement Procedure

On the **Home** screen, select the **OD600** tab and click **OD600**.

If necessary, specify the cell count conversion factor and a secondary monitoring wavelength or absorbance correction.

Pipette **2 µL of blank solution** (i.e., the culture medium of the target cell suspension) onto the lower pedestal, then lower the measurement arm, **or** insert a blank cuvette into the cuvette holder.

Tip: If using a cuvette, ensure that its optical path is aligned with the instrument's optical path.

Click **Blank** and wait for the measurement to complete.

Raise the measurement arm and wipe the upper and lower pedestals with a new piece of laboratory lint- free tissue, or remove the blank cuvette.

Pipette **2 µL of the sample solution** onto the pedestal, then lower the measurement arm, **or** insert the sample cuvette into the cuvette holder.

Begin sample measurement:

Pedestal: Lower the measurement arm and click **Measure**.

Cuvette: Click **Measure**.

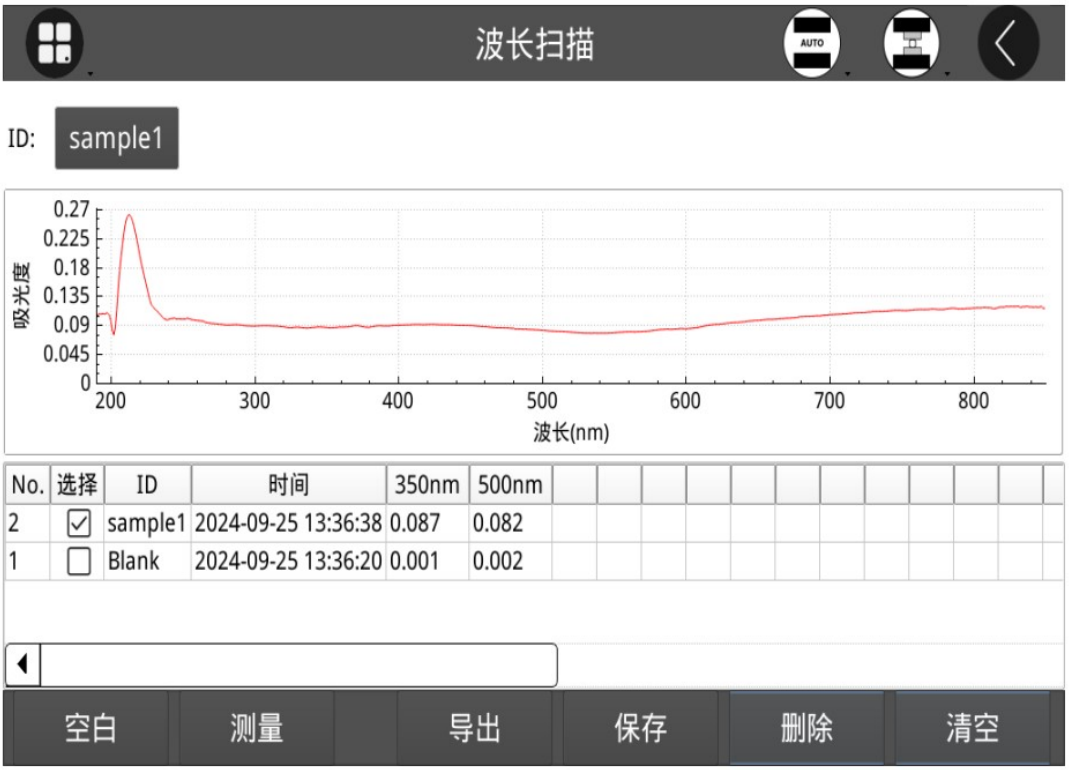
Once the measurement is completed, the spectrum and reported values will be displayed.

When the experiment is finished, raise the measurement arm and wipe the pedestals with a new lint-free tissue, or remove the sample cuvette.

Click the **Return** button (upper right) to go back to the main interface, or click **Export** to export the experimental data, or **Save** to store the results.

⚡ If you'd like, I can also turn this into a **step-by-step flowchart or structured Excel table** (like we did with your hyperspectral specs) for easier use in your manuals. Do you want me to prepare that?

2. UV-Vis Full Wavelength Scan



Procedure:

On the **Home** screen, click **UV-Vis** to enter the full wavelength scan interface.

Specify up to **40 monitoring wavelengths** (you may also define them later if needed).

Click the grid icon in the upper left corner to select options and enter the settings interface.

In the settings interface, specify the wavelengths to be measured.

Available modes:

A – Absorbance

T – Transmittance

E – Intensity

Select according to your measurement needs

Pipette **1–2 µL of blank solution** onto the lower pedestal, then lower the measurement arm, **or** insert a blank cuvette into the cuvette holder.

Tip: If using a cuvette, ensure its optical path is aligned with the instrument's light path.

Click **Blank** and wait for the baseline measurement to complete.

Raise the measurement arm and clean both the upper and lower pedestals with a new lint-free tissue, or remove the blank cuvette.

Pipette **1–2 µL of sample solution** onto the pedestal, then lower the measurement arm, **or** insert the sample cuvette into the cuvette holder.

Start sample measurement:

Pedestal: Lower the measurement arm and click **Measure**.

Cuvette: Click **Measure**.

After measurement, the spectrum and report values will be displayed.

When finished, raise the arm and clean the pedestals with a new lint-free tissue, or remove the sample cuvette.

To complete the experiment:

Click the **Return** button (upper right) to go back to the main screen,

Or click **Export** to output the experimental data,

Or click **Save** to store the experimental results.

Kinetics Measurement

The instrument can be used for time-based kinetic detection of samples in a cuvette. Up to **four wavelengths between 190 nm and 850 nm** can be specified for continuous absorbance monitoring at user-defined intervals, across up to six stages.
Cuvette measurements provide an extended lower detection limit.

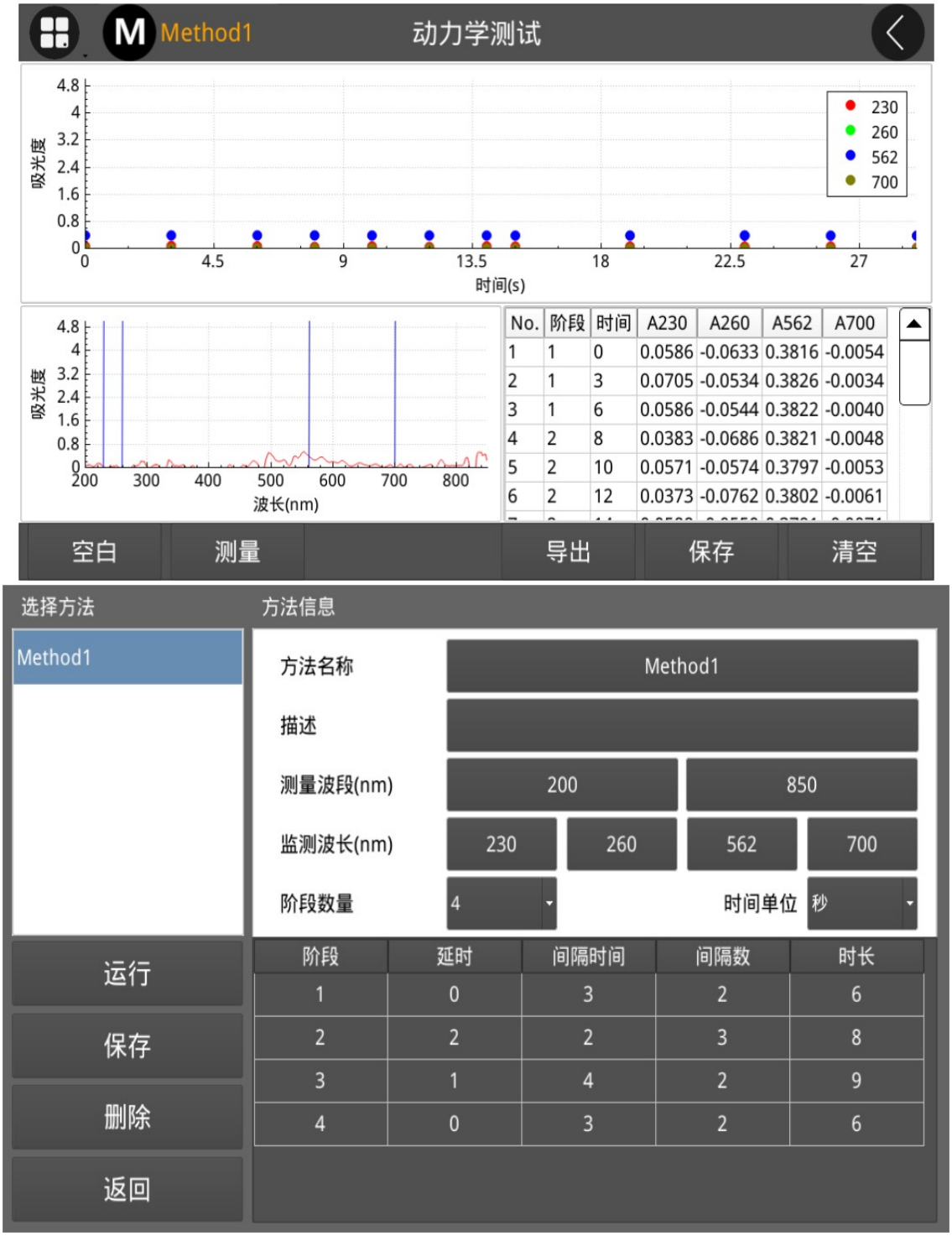
Note: During cuvette measurement, the cuvette lid may be lifted if reagents need to be added to the sample solution.

Procedure:

On the instrument **Home** screen, select **Kinetics Measurement**.

The **Kinetics Setup** screen will appear.

If the currently selected data storage location contains one or more kinetic methods, they will be listed in the **Select Method** box.



1. Select a method:

Click the square button in the upper left corner and select "Options", or click the M button directly to enter the Settings interface.

According to customer needs, you can set up a maximum of 6 stages of steps. Set parameters such as delay, interval time and interval number in sequence. Click "Save" after setting.

Note: If the optical path of the cuvette is not 10 mm, pay attention to the conversion.

2. Click Run.

3. Perform blank detection:

-Add enough blank detection solution to cover the optical path of the instrument in a clean, dry cuvette.

-Lift the instrument detection arm and insert the blank detection cuvette into the cuvette holder to ensure that the light path of the cuvette is aligned with the light path of the instrument.

-Click "blank".

-Wait for the blank test to be completed, then remove the cuvette.

4. Test samples:

-Add enough sample solution to cover the optical path in a clean, dry cuvette.

-Insert the sample cuvette into the cuvette holder and ensure that the light path is aligned.

-Click "Test".

Note: You can add reagents to the sample solution at any time during the test.

During the operation, you can click "Stop" to end the experiment in advance.

-Wait for all test phases to complete.

-Take out the colorimetric dish and clean it according to manufacturer specifications.

The results of each test are displayed in real time. After all the stages are completed, the spectrum and reported values of the whole experiment are displayed.

5. After completing the data check, click the upper right corner to return to the main interface or click the "Export" button to export the data of the experiment, or click the "Save" button to save the data of the experiment.

1 maintenance instruction

8.1 maintenance overhaul

• Clean the base with deionized water

For Microdrop2000 spectrophotometer, the most important maintenance requirement is to keep the base surface clean. After each measurement, clean the base with clean dust-free paper as soon as possible to avoid accumulation of sample residues. After completing the last sample measurement, it is recommended to thoroughly clean and wipe the base with deionized water.

8.2 maintenance routine

8.2.1 Clean the touch screen

To avoid permanent damage to the touch screen, do not clean the touch screen with abrasive materials (such as paper towels), do not apply excessive pressure, and do not spray liquid directly on the touch screen.

To clean the touch screen, gently wipe it with a soft non-fuzzy cloth (e.g., microfiber). If necessary, use a glass LCD cleaner and follow the manufacturer's instructions.

8.2.2 Maintenance of the base

The base needs to be maintained regularly to maintain the integrity of the detection. The following provides a timeline and procedure for cleaning and repairing the base.

8.2.2.1 Clean the base

To avoid residue and cross-contamination, clean the base before the first blank test or sample test and after each test. Regular maintenance may require additional cleaning (as follows) or repair.

Consumables required

No-lab dust-free paper, deionized water (DI H2 O), suitable for thorough cleaning: 0.5M HCl

Clean the base between tests:

Lift the instrument test arm and wipe the upper and lower bases with new laboratory dust-free paper.

Clean the base when rotating users:

1. Lift the detection arm and wipe the upper and lower bases with the new laboratory dust-free paper

2. Add 3-5 μL DI H2O to the lower base.

3. Lower the detection arm and wait 2-3 minutes.

4. Lift the detection arm and wipe the upper and lower bases with new dust-free paper.

Note: When thorough cleaning is required (e.g., removal of dry samples remaining on the base), use 0.5M HCl Replace the DI H2O in the above procedure, and then use 3-5 μL DI H2O.

8.2.2.2 Repair the base

The base surface may gradually lose its "adjusted" properties over time, particularly after exposure to solutions containing isopropanol or surfactants/detergents (such as Bradford's reagent). An unadjusted base can cause droplets beneath it to flatten, hindering proper liquid column formation when the detection arm descends. This may result in spectra appearing "rough" or showing "sawtooth" patterns.

If the sample flattens on the base (rather than forming "microbeads" or circular droplets) or if the column breaks during the test period, repair the base.

Follow these steps:

1. Lift the instrument detection arm and transfer 3 μL 0.5M HCl to the lower base.

2. Lower the detection arm and wait 2-3 minutes.

3. Lift the detection arm and wipe the upper and lower bases with the new laboratory dust-free paper

4. Add 3 μL DI H2O to the bottom base.

5. Lower the detection arm and wait 2-3 minutes.

6. Lift the detection arm and wipe the upper and lower bases with new dust-free paper.

Note: When you wipe the upper base, support the instrument probe arm with one hand to avoid damage to the probe arm.

7. Fold a clean piece of lab dust paper into quarters and then use it to vigorously wipe each base surface at least once 50 times.

8. Use a can of air to remove any paper scraps left on the base.

8.3 Instrument decontamination

If biological contamination needs to be removed, wipe the sample with freshly prepared bleach (e.g., 5.25% sodium hypochlorite)

The platform ensures that no bioactive material is present on the sampling platform. The metal parts of the machine are made of stainless steel and are resistant to common laboratory solvents

Decontaminate the instrument after testing samples containing hazardous substances and before returning the instrument to us for maintenance or repair.

Note: If your instrument needs maintenance or repair, please contact us or your local distributor.

8.3.1 Decontamination of the base

1. Lift the instrument test arm and wipe the upper and lower bases with new laboratory dust-free paper.

2. Transfer 2-3 μL of diluted bleach solution (see the consumables required) to the lower base.

3. Lower the detection arm and wait 2-3 minutes.

4. Lift the detection arm and wipe the upper and lower bases with new dust-free paper. Add 3-5 μL DI H2O to the lower base.

5. Lower the detection arm and wait 2-3 minutes.

6. Lift the detection arm and wipe the upper and lower bases with new dust-free paper.

8.3.2 Decontamination of instrument surface

1. Moisten a clean soft cloth or laboratory dust-free paper (see consumables required) with diluted bleach solution and gently wipe the exterior of the instrument.

2. Use DI H2O to remove the bleach solution with a clean soft cloth or dust free paper.

8.4 Maintain the cuvette sampling system

Clean and dry the cuvettes after each test. Use a scratch-free cuvette and avoid fingerprints that may affect the results.

When the instrument is not in use, please turn off the instrument detection arm.

Use ear balls or canned air to remove any dust from the plate rack.

Use the new laboratory dust-free paper to clean up any spills in the plate rack.

To clean and maintain the cuvette, follow the manufacturer's recommendations.

8.5 Parts that may need to be replaced

Typically, the only component requiring regular replacement is the flash. The Microdrop2000's flash is designed to theoretically handle 300,000 shots. The remaining lifespan of the flash cannot be detected. When the flash approaches its end, its output becomes highly unstable or completely extinguishes. In such cases, contact your local authorized service provider to have the flash replaced.

2 matters need attention

9.1 Operational precautions

Do not remove the instrument cover. Removing the cover exposes the operator to sharp edges and fragile fiber optic cables. Removing the cover will invalidate the warranty of the instrument.

The Microdrop2000 spectrophotometer is designed for indoor operating environments that meet our requirements.

Follow the following precautions during use to avoid damage to your spectrophotometer:

Use a grounding power cord that is suitable for your electrical equipment. If the power cord included is incompatible or damaged, contact us.

LCD screens are made of chemically tempered glass that has been heat treated to be strong and durable. However, if the screen cracks or breaks, please contact us for replacement.

Use a solvent compatible with the instrument

Do not use hydrofluoric acid (HF) on the base. Fluoride ions will permanently damage quartz fiber optic cables.

To prevent damage caused by leakage, keep the liquid container away from the instrument.

Do not use a jet or spray bottle near the instrument, as the liquid may flow into the instrument and cause permanent damage.

9.2 Preparation for use

Failure to use the instrument in the manner specified in the accompanying documentation may weaken the protective function provided by the instrument.

Only perform the procedures described in this document. If any other problems occur, contact us. Any other maintenance work must be performed by trained personnel.

Take care not to remove the instrument cover. All maintenance of the instrument must be performed by trained personnel.

When the system is delivered, check for any damage marks on the exterior of the shipping box. If any damage occurs, please contact us or your local distributor for instructions on how to handle this situation.

The shipping container must be moved to the installation site at least 24 hours prior to installation.

Within the shipping container, seal the instrument in a plastic bag to ensure it is dry. Before opening the plastic bag, let the instrument stand at room temperature for 24 hours.

If the plastic bag is opened before the instrument reaches room temperature, moisture will condense on the optical components and cause permanent damage.

Note to keep the instrument upright at all times.

The warranty will not cover:

Damage caused by improper handling techniques.

Damage caused by removing the sealed plastic bag before the instrument reaches room temperature.

Note: All system support facilities must be installed before the instrument is delivered. Support facilities must comply with all local building and safety regulations.

9.3 Lift or carry the instrument

To avoid personal injury, use appropriate lifting techniques when moving instruments or other system components.

9.4 Electrical requirements and safety

The system's power supply must originate from a dedicated uninterruptible power supply (UPS). There should be no voltage fluctuations, transient spikes, frequency deviations, or other line disturbances that could compromise reliability. If you suspect power quality issues at your location or plan to install the system in a heavy industrial environment, we recommend conducting a power quality check prior to installation or preparing an UPS uninterruptible power supply.

Only qualified personnel are allowed to use appropriate testing equipment to test the voltage, current and frequency of the line.

Only service representatives who have been trained and certified by us are allowed to repair parts with this symbol.

If the protective cover of a system component is damaged, close the system and lock it to avoid any unnecessary operation. After shipment, be sure to check the protective cover to see what transportation stress has been applied.

Even if all power to the instrument is disconnected, the capacitor may remain charged for up to 30 seconds, which can cause electric shock.

Do not allow liquid to flow over or into any surfaces that may enter the interior of the instrument.

Do not attempt to remove the instrument cover.

Each outlet used must be equipped with a grounding wire. The grounding wire must be a current-free wire connected to the ground wire in the main distribution box.

9.5 power line

Ensure that the appropriate grounding power cord is used for your electrical equipment. If the power cord received is not suitable for your local power system, or if the power cord is damaged, contact us.

Transmission line adjustment accessories

In the event of a power outage elsewhere in the building, the UPS reduces the chance of

system shutdown.

9.6 Fire safety and burn hazards

Do not place the instrument in a place where it is difficult to operate the power switch or access the power supply or power cord.

To avoid burns and fire or explosion:

Extra care must be taken when testing combustible or explosive samples.

Do not block any ventilation holes on the instrument or its power supply.

Use only the correct replacement power supply provided by us.

9.7 Optical safety

The instrument is designed with a protective cover to prevent users from ultraviolet light exposure.

Warning: To avoid personal injury, do not look directly at the light.

9.8 hazardous substance

9.8.1 Compatible solvents

Many standard spectroscopic methods are based on the use of a solvent. Others involve corrosive or pressurized samples in a gaseous state.

Do not place volatile solvents or flammable samples near the instrument.

Ensure proper ventilation in the work area.

Solutions commonly used in biological laboratories for dissolving or diluting samples are generally compatible with the fiber optic base of the Microdrop2000 spectrophotometer. However, certain solvents with high vapor pressure may not be conductive during micro-volume measurements using the base's detection system. If your sample exhibits high vapor pressure characteristics, it is recommended to use instruments equipped with cuvette detection capabilities.

All hydrofluoric acid (HF) and strong acids and bases are not suitable to avoid damage to the quartz surface of the fiber.

The following solvents are compatible for use on the Microdrop2000 base.

Note: If these solvents spill on any surface other than the base, the instrument may be damaged.

Methanol, isopropanol, ether, DMSO, THF, benzene, dilute hydrochloric acid, ethanol, butanol, chloroform, DMF, toluene, sodium hydroxide, dilute HNO3, n-propanol, acetone, carbon tetrachloride, acetonitrile, hexane, sodium hypochlorite (bleaching agent), dilute acetic acid.

It is recommended to erase all corrosive solvents from the base immediately after testing.

In addition, it is recommended that users perform a series of DI H2O sample tests at the end to ensure that no solvents are accidentally left on the base.

9.8.2 Biological hazards or radioactive and infectious substances

Biological samples, such as human and other animal tissues, bodily fluids, infectious agents, and blood, may pose potential risks of transmitting infectious diseases. Wear appropriate protective equipment. Before handling potentially infectious materials, personnel should receive training in accordance with applicable regulations and institutional requirements. When operating or handling these materials, strictly follow your institution's "Biosecurity Plan" protocol.

Warning: Reduce the risk of exposure to potentially infectious samples:

Do not allow the sample to spill onto any instrument components.

If spillage occurs, be sure to disinfect the external surfaces immediately in accordance with your laboratory operating procedures.

If instruments, accessories, components, or other related materials are contaminated by biological hazards, radioactive substances, infectious materials, or any other substances and/or situations that may pose health risks or physical harm to employees, they should not be disposed of as waste or returned to us or other accessory manufacturers. If you have any questions about the requirements for decontamination, please contact us.

3 frequently asked questions

10.1 Factors affecting measurement results

10.1.1 Sample overlap

If the sample is not wiped clean and remains on the loading platform, it may cause overlapping measurements, leading to biased results. Therefore, immediately wipe off samples from both the upper and lower loading platforms after each measurement. For concerns about residual contamination, clean the loading platform with 2.5 μl of pure water after measuring high-concentration samples. After multiple measurements, thoroughly clean both loading platforms.

10.1.2 Sample uniformity

Various measuring instruments, including the traditional spectrophotometer, are prone to large data differences when measuring samples that are not uniformly mixed (especially when the sample volume is small). Therefore, the samples must be thoroughly mixed before sampling.

10.1.3 Evaporation effect

The evaporation of the sample during measurement will cause the concentration of the sample to increase by 1-2% compared with the true value. High volatile solvents, such as hexane, are easy to evaporate before the measurement is completed. It is best to use low

volatile solvents, such as DMSO or pure water, to avoid measurement failure.

10.2 The problem of sampling accuracy and repeatability

If the repeatability of the test results is poor or the difference with the theoretical value is large, it is likely that the sample platform is not clean, the mixing of the sample is not uniform enough, or the two platforms do not form a vertical column of liquid due to the small amount of sampling. The following methods can be used to improve this phenomenon:

Before starting to use the control software, make sure that the surface of the sample platform is clean to avoid getting a wrong absorbance value during measurement

(which may even be negative) and incorrect signal saturation. Therefore, develop good measurement habits and, after completing the measurement of the last sample, remove any contaminants that may have occurred on the platform with pure water.

If the sample fails to form a vertically aligned liquid column during measurement, abnormal results may occur. A sufficient sample volume (0.5-2 μl) is required to ensure a well-formed liquid column between the two platforms. Protein solutions typically exhibit low surface tension, which makes it difficult to establish such a column. In such cases, add some pure water to the loading platform and close the mirror. After 2-3 minutes, wipe both platforms with clean lint-free paper. Then, fold the dry paper several times to increase its thickness, and vigorously wipe the loading platform 15-20 times to enhance surface tension between the platform and the liquid sample, achieving optimal measurement results.

Before measurement, the DNA sample was heated to 55℃ and thoroughly mixed by shaking before sampling. Since Microdrop2000 requires only a very small volume of sample, the uniformity of the sample is crucial. According to the results, for larger molecular samples,

For example, genomic DNA or lambdaDNA is particularly prone to this phenomenon.

Note: Generally, spectrophotometers using colorimetric tubes have no obvious effect due to large sample volume and uneven sample.

Make sure that the blank solution (blank) and the solvent of the sample are the same thing to avoid getting a wrong absorbance value.

The concentration of the sample should not be too low. When the concentration is close to the limit of the measurement range, the absorbance value of the sample is likely to change significantly and affect the calculation of the concentration. Please refer to each chapter to confirm the measurement range of various samples.

10.3 Unusual absorption spectrum

If the absorption spectrum shows a normal shape but multiple jagged areas appear in the curve, the measured values at the jagged areas are unreliable, while the measured values in the smooth areas of the curve are still accurate. This phenomenon may be caused by a dirty sampling platform. Simply clean the sampling platform and restart the software.

Appendix A Instrument specifications

1、technical parameter

1. Scope of application: can be used for nucleic acid concentration and purity detection, also suitable for a variety of protein concentration determination, can also be used for cell and bacterial concentration detection and routine full wavelength scanning;

2. Light source: imported xenon flash;

3. Wavelength range: 190-850 nm continuous wavelength full spectrum analysis;

4. Optical path: optical path 0.02 mm, 0.2 mm, 1 mm, can be automatically matched to the best optical path according to the sample concentration, no manual setting;

5. Light absorption range: base 0-750A (10mm optical path);

6. Detection range (dsDNA): 2 ng/μL ~ 37500 ng/μL;

7. Light absorption accuracy: 3% (at 0.97A at 302 nm);

8. Spectral resolution: ≤2.4 nm (FWHM at Hg 254 nm);

9. Wavelength accuracy: ±1nm;

10. Detection repeatability: 0.002A (1.0mm optical path) or 1%CV;

11. Sample detection and data processing time: less than 4 seconds per round;

12. Detector type: 2048-CMOS array;

13. Equipped with magnetic light shield plate to minimize the interference of ambient light and the effect caused by liquid evaporation. After removal, it is easy to observe the liquid column situation;

14. The loading sample point is made of 303 high polished and high wear-resistant stainless steel, which is integrated with the main engine, and can be directly sampled and tested;

15. Detection mode: there are two modes of base detection and cuvette detection at the same time;

16. Minimum sample volume: 1 μL;

17. Supported applications: Nucleic acid detection (A260, A260/A280, A260/A230) and oligonucleotide concentration; Protein detection (Bradford, BCA, Lowry, Pierce 660, custom proteins); OD600 bacterial solution detection; UV-Vis full-wavelength scanning and kinetic measurements;

18. Protein concentration detection: The software has a variety of different protein concentration detection methods, suitable for the needs of different customers, including:

1Abs=1 mg/ml, BSA, IgA, Lysozyme, molar extinction coefficient method, percentage extinction coefficient method and custom;

19. Built-in 7-inch touch screen, no need for an external computer, can be operated directly on the touch screen, compatible with laboratory gloves;

20. Operating system and configuration: Linux, CPU: ARM Cortex-A7 processor, storage space: 32GB;

21. Operation interface: Chinese and English dual interface, can be freely switched.

22. Data show that the OD value, ratio and concentration are directly displayed in the form of table in the operation interface, so as to carry out intuitive comparison and analysis between samples.

23. The image and table storage mode is adopted, the table is compatible with excel, which is convenient for subsequent data processing, the screenshot function is supported, and the data can be saved in the device or exported to a USB drive.

24. Power adapter: input 110~220VAC, output 24V DC 3A, power consumption: 72W

25. Size (W×D×H) 235×295×200 mm

26. Weight: 5kg

2、location of apparatus

1. Ultramicro UV-Vis spectrophotometer host 1 set. 2. Set of instrument power adapter. 3. Supporting detection software 1 set.

4 A user manual. 5. A certificate of conformity. 6. A warranty card. 7. An acceptance form. 8. A packing list.

Appendix B Absorbance and concentration calculations

From Lambert-Beer (Lambert-Beer) law, we can find that there is a certain relationship between absorbance and concentration:

$A = -log（I/I0）=-lgT=klc$

Where:

A——— Absorbance of the substance;

I0——— Intensity of incident monochromatic light;

I——— The intensity of transmitted monochromatic light; T——— The transmission ratio of the substance;

——— Absorption coefficient of matter;

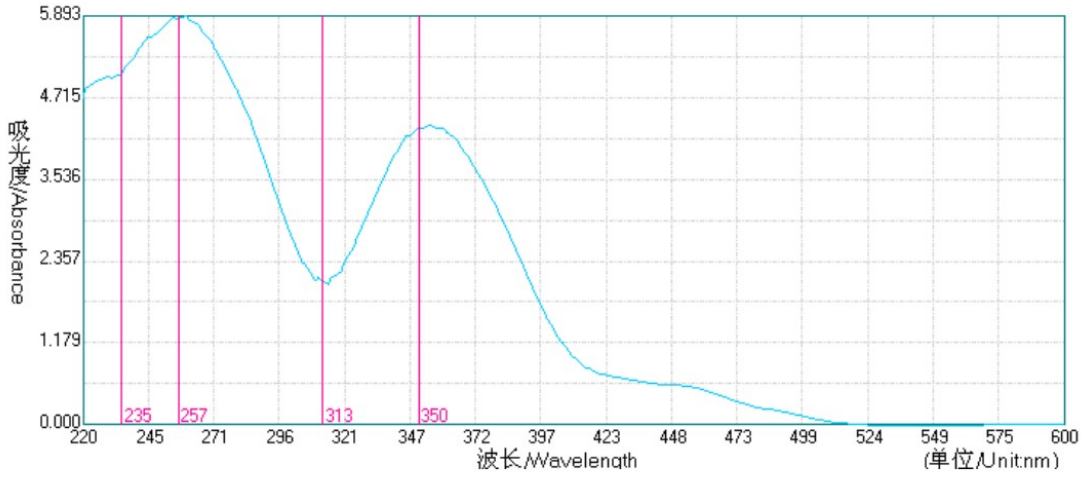
——— The optical path of the substance being analyzed;

c——— The concentration of the substance.

When the unit of c is g/L and that of l is cm, then A=klc. The proportionality coefficient k is called "gram molecular absorption coefficient" and has the unit of L/g·cm. The molar absorption coefficient ε is obtained by multiplying MW (molecular weight) by a.

When the unit of c is mol/L and the unit of l is cm, then A=εlc, and the proportionality coefficient ε is called "molar absorption coefficient", with the unit of L/mol ·cm.

Appendix C Test curve of potassium dichromate solution

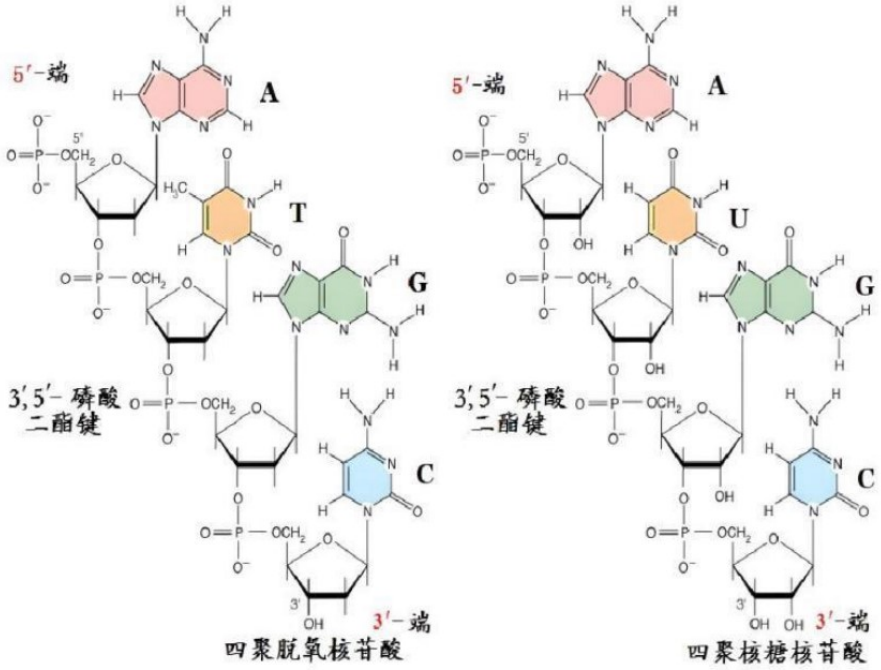


Appendix D. Principles of oligonucleotide concentration measurement

1. brief introduction

Oligonucleotides are short chains of nucleic acids composed of deoxyribonucleotides or ribonucleotides. They are short chains of nucleic acids containing about 20 to 30 bases and can be used in DNA sequencing, PCR amplification, genetic testing, antisense nucleotide research and other fields.

2. background knowledge



构成DNA和RNA核苷酸的结构和连接方式

Nucleotide is the basic unit of nucleic acid macromolecule. The hydroxyl group of pentose and phosphate in nucleoside are combined by phosphoric ester bond to form nucleotide. Most nucleotides in organisms are formed by phosphorylation of hydroxyl group on the fifth carbon atom of ribose or deoxyribose.

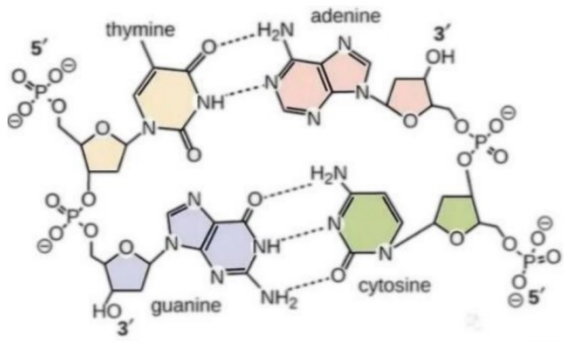
The connection between nucleotides is formed by the hydroxyl group (C-3 ') at the third carbon atom of one nucleotide binding to the 5' -phosphate of another nucleotide, creating a 3 ',5' -phosphodiester bond. This linkage determines the specific directionality of nucleic acid chains, with each strand having two distinct termini: the 5 '-end features a free phosphate group at the C-5' position, while the 3 '-end contains a free hydroxyl group at the C-3' position.

RNA: Phosphoryl ribose containing nitrogenous bases (base types include A: adenine; U: uracil; C: cytosine; G: guanine)

DNA: Phospho-deoxyribonucleotide containing nitrogenous bases (base types include A: adenine; T: thymine; C: cytosine; G:

guanine）

In addition to phosphodiester bonds, hydrogen bonding interactions exist between nitrogen bases in nucleic acid chains. These interactions are formed through the interaction of hydrogen atoms between nitrogen bases and electron pairs between nitrogen atoms. Such interactions enable nucleic acid chains to adopt highly distinctive spatial configurations. A prime example is the DNA double helix structure, which is created by two complementary nucleic acid strands interacting via hydrogen bonds.



The bases are paired in the following way: A-T A-U (two hydrogen bonds); C-G (three hydrogen bonds).

In terms of spatial configuration, DNA is a double-molecular structure formed by two long chains entwined into a twisted shape. The structure is stable and convenient for storing genetic information, while RNA is mostly single-stranded and generally only serves as a carrier of genetic information during transcription and translation.

3. Principle of oligonucleotide concentration calculation

3.1 Lambert-Beer Law

From Lambert-Beer law, we can find that there is a certain relationship between absorbance and concentration:

$A=\varepsilon \times c \times p$

A is the absorbance value, expressed in A;

ε Represents the wavelength-based molar extinction coefficient (or molar absorption coefficient), in units of L ·mol-1 ·cm-1;

dRepresents the molar concentration, in units of mol/L or molarity (M);

Represents the optical path length, in cm.

Therefore, to know the concentration of the sample (unit: ng/μL), in addition to the measured absorbance and the known optical path length, we also need to know the molar extinction coefficient ε (unit: L ·mol-1 ·cm-1) and the molecular weight MW (unit: Da, full name Dalton

(Dalton)， 1Da= 1g/mol）

3.2 Molar extinction coefficient of oligonucleotides

Oligonucleotides are short nucleic acid chains composed of deoxyribonucleotides or ribonucleotides, referred to as oligonucleotide DNA and oligonucleotide RNA respectively. These approximately 20-30 base-length nucleotide chains are commonly used as probes to determine the structure of DNA or RNA. They serve in DNA sequencing, PCR amplification, genetic testing, antisense nucleotides, and gene core applications

In the fields of single strand, electrophoresis, fluorescence in situ hybridization and so on. However, there is no strict rule on the number of nucleotide residues when using this term. In many literature, polynucleotide molecules containing 30 or more nucleotide residues are also called oligonucleotides.

When base composition is random, the optical density (OD) of double-stranded DNA can be approximated as 50 μg, while that of single-stranded DNA is 33 μg. However, for custom-designed oligonucleotide chains, calculations must be performed based on specific sequences. This is because even identical base quantities with different arrangements may result in varying absorbance coefficients. Furthermore, variations in base composition can also lead to significant differences in absorbance values.

Since the extinction coefficient depends on the precise nucleotide composition and sequence, it should take into account both the absorbance of each different base nucleotide and the effects between adjacent nucleotides. There are several ways to determine the extinction coefficient of an oligonucleotide

The nearest neighbor method (commonly translated as the nearest neighbor method or proximity method) is more accurate than the method of summing up the extinction coefficients of individual nucleotides. This device uses the nearest neighbor method for calculation.

Please note that even when using the "nearest neighbor" method to calculate extinction coefficients, the results may still differ from the true values by up to 10%. Additionally, these coefficients can be influenced by factors such as buffer type, ionic strength, and pH value. It should be particularly emphasized that even in neutral environments, the absorption peaks and troughs corresponding to different bases may not necessarily align at 260nm and

The absorption peak and trough of oligonucleotides at 230nm are not limited to 260nm and 260nm, as they can also occur at 230nm due to minor but real displacement influenced by pH values. Similarly, the A260/A280 ratios of five independent nucleotides are: guanine 1.15, adenine 4.50, cytosine 1.51, uracil 4.00, and thymine 1.47. For specific nucleic acid sequences, the A260/A280 ratio essentially equals the weighted average of individual bases. The conventional A260/A280 and A260/A230 ratios used for pollutant detection are highly dependent on base composition, making them unsuitable for oligonucleotides.

The following formula is used to calculate the molar extinction coefficient of a specific oligonucleotide base sequence.

$$\varepsilon_{260} = \sum_{1}^{N-1} \varepsilon_{Nearest\ Neighbor} - \sum_{2}^{N-1} \varepsilon_{Individual\ Bases} + \sum_{1}^{N} \varepsilon_{Modifications}$$

ε 260 refers to the extinction coefficient at 260nm.

ε Nearest neighbor refers to the extinction coefficient of the nearest neighbor.

ε Individual bases refers to the extinction coefficient of a single base.

ε Modifications refers to the extinction coefficient changes caused by various modifications such as fluorescent dyes. This paper does not involve this index for the time being. For more detailed and accurate calculation, please refer to the calculation tools of major primer synthesis companies.

Absorption coefficient of DNA and RNA oligonucleotides [unit: L·mol-1·cm-1].

These parameters were obtained at 260nm under neutral pH conditions. The calculated extinction coefficient error was approximately 4% (260 nm, neutral pH conditions).

DNA		RNA	
ribotide	coefficient of light extinction	ribotide	coefficient of light extinction
pdA	15400	pA	15400
pdC	7400	pC	7200
pdG	11500	pG	11500
pdT	8700	pU	9900
dApdA	27400	ApA	27400
dApdC	21200	ApC	21000
dApdG	25000	ApG	25000
dApdT	22800	ApU	24000
dCpdA	21200	CpA	21000
dCpdC	14600	CpC	14200
dCpdG	18000	CpG	17800
dCpdT	15200	CpU	16200
dGpdA	25200	GpA	25200
dGpdC	17600	GpC	17400
dGpdG	21600	GpG	21600
dGpdT	20000	GpU	21200
dTpdA	23400	UpA	24600
dTpdC	16200	UpC	17200
dTpdG	19000	UpG	20000
dTpdT	16800	UpU	19600

case：

Take 5 '-ATG C-3' as an example

ε= (dApdT+dTpdG+dGpdC) -(pdT+pdG) L·mol-1·cm-1

ε= (22800+19000+17600) - (8700+11500) L·mol-1· -1 cm

ε= 39200 L·mol-1·cm-1

3.3 Oligonucleotide molecular weight

Another important data that appears on the primer synthesis sheet is the molecular weight MW. The molecular weight of an oligonucleotide is the compound per mol

The mass of (6.02×10^{23} molecules) is the total molecular weight of all atoms that an oligonucleotide may contain. Molecular weight data is used to convert OD260 units into mass units, and the molecular weight of an oligonucleotide generally refers to that of its free acid form.

Relative molecular mass (Mr)is the sum of the relative atomic masses of atoms in a chemical formula, denoted by the symbol Mr.While numerically equivalent to molar mass, they differ in units: relative molecular mass is measured in "1", whereas molar mass is measured in g/mol. Molar mass refers to the mass of a substance per unit amount, represented by the symbol M. When expressed in mol units, molar mass is measured in g/mol, numerically equal to the atomic or molecular mass of the substance.

For a given compound, its molar mass is fixed. The mass of a substance varies with the amount of the substance.

The molecular weight of oligomers is the sum of the weights of individual bases and chemical modifications. Oligomer synthesis typically does not contain the 5-phospho group, which must be subtracted. Here PO2H=63.9805 and H2=2.0159

Molecular scale				
Oligonucleotide DNA components	dA	dC	dG	dT
formula weight	313.2095	289.1845	329.2089	304.1962
Oligonucleotide RNA components	A	C	G	U
formula weight	329.2089	305.1839	345.2083	306.1687

Note: Note that the molecular weight corresponding to a water molecule that would be lost after conjugation has been removed.

DNA, molecular weight (specifically synthetic DNA primers. The calculation assumes no single phosphate at the 5' end)

Molecular weight MW = NA × 313.2095 + NC × 289.1845 + NG × 329.2089+ NT × 304.1962-

61.9646

Here, NA, NC, NG, and NT represent the quantities of A, C, G, and T in an oligonucleotide DNA. -61.9646=-PO2H + H2 =

-63.9805+2.0159。

Molecular formula: (in the order of ACGT) C10H14N5O6P; C9H14N3O7P; C10H14N5O7P; C10H15N2O8P;

The calculation formula of RNA molecular weight is:

The molecular weight MW is calculated as NA × 329.2089 + NC × 305.1839+NG × 345.2083 + NU × 306.1687-61.9646

Here NA, NC, NG and NU respectively represent the number of A, C, G and U in an oligonucleotide RNA. Note that ACG in RNA and ACG in DNA do not represent the same substance, so the corresponding molecular weights are different.

It should be noted that the molecular weight calculated by the aforementioned formula is an approximate value, as oligonucleotide molecules may be influenced by other factors such as phosphate groups and methylation in real-world applications. For longer oligonucleotide molecules, the mass of phosphate groups must also be considered during molecular weight calculation. In precision experimental scenarios, techniques like mass spectrometry are typically employed to measure the molecular weights of oligonucleotide molecules.

case：

Still taking the 5 '-ATG C-3' as an example

Molecular weight =1 × 313.2095 + 1 × 289.1845 + 1 × 329.2089) + 1 × 304.1962-61.9646=1173.8345

3.4 Oligonucleotide concentration calculation

In primer synthesis, there is a common indicator OD, sometimes also written as O.D or OD260, which represents the total amount of primer, but the unit is neither g nor mol. Let's take a closer look at it.

First, OD stands for optical density. Some literature describes two measurement methods: A (absorbance) and T (transmittance). Here, A (absorption) is the absorbance value

denoted as ABS, while T (transmittance) represents the transmittance. The relationship between T and A is expressed as lg(A/B). Other studies suggest that OD and ABS have equivalent meanings, i.e., OD=ABS= -lg(T), though in specific industries like DOAS1, the logarithmic form OD= -ln(T) is adopted.

1. *DOAS: Differential Optical Absorption Spectroscopy (DOAS) is a kind of spectral monitoring technology. Its basic principle is to use the narrow band absorption characteristics of gas molecules in the air to identify gas components, and deduce the concentration of trace gas according to the intensity of narrow band absorption.*

As described above, OD is a ratio that does not represent the actual amount of substance. However, in the primer synthesis industry, OD has become a standardized unit of measurement. Specifically, it refers to the concentration of primers in a solution when the absorbance value reaches 1 at 260nm wavelength under 1cm optical path length (1 mL solution). The formula for 1 OD is 1 OD= ε · c · p, where ε represents the molar extinction coefficient of the primer, measured in units of..

II·mol-1 ·cm-1, c is the concentration, unit is mol/L, p is 1cm at this time.

c=A/(ε* p)

Still taking 5 '-ATG C-3' as an example, assuming the absorbance is 1 and p is 1cm, then

c=A/(ε* p) =1/（39200 L ·mol-1 ·cm-1 * 1cm）=2.551*10-5mol/L=25.52nmol/L。

This value is generally described as nmole/OD260nm (1ml sample) or nmole/OD260, which refers to the corresponding molar concentration when the absorbance value of 1ml of the oligonucleotide at 260nm under 1cm light path is measured.

Convert to mass concentration

C=2.551*10-5mol ·L-1*1173.8345g ·mol-1=29.94*103g/L=29.94ng/μL

This value is generally described as μg/OD260nm (1ml sample) or μg/OD260, which refers to the corresponding mass concentration when the absorbance value of 1ml of the oligonucleotide at 260nm under 1cm path length is measured.

Appendix E Protein quantification method introduction

Proteins are crucial biological macromolecules with diverse types, varying structures, and significant molecular weight differences. Their varied functions present substantial challenges in developing ideal and universal protein quantification methods. Currently, multiple approaches exist for determining protein content. Below are some protein measurement techniques developed based on different protein properties:

Physical properties: UV spectrophotometry

Chemical properties: Kjeldahl nitrogen determination, double urea method, Lowry method, BCA method, colloidal gold method

Dyeing properties: carma's brilliant blue staining method, silver staining method

Other properties: Fluorescence method

Table I Comparison of commonly used methods for determining protein content

method	measurement range (μg/ml)	variety classes The difference in protein different	Maximum absorption wavelength (nm)	merit	shortcoming
Kjeldahl determination	——	small	——	The results are most accurate and are often used for standard determination	The operation is troublesome, time-consuming, low sensitivity, easy to be interfered by other nitrogen-containing substances (such as tricyanamide)
ultraviolet spectrophotometry	100~20000	big	280 205	Sensitive, fast, no sample consumption	Nucleic acid substances have an effect, and different proteins are not comparable
The biocyanuric acid method	1000~10000	small	540	Good repeatability and linear relationship	Low sensitivity, narrow measurement range, large sample amount required
Protein Loughry method (Folin-phenol test The method of	20~500	big	650 750	sensitive	It takes a long time and has many interfering substances

the agent)					
Coomassie brilliant blue G-250 (Bradford Act)	50~500	big	595	Quick response, simple operation, less sample consumption	The difference between different proteins is large, and the standard curve line is poor, which is easy to be interfered by detergent
BCA law	50~500	big	562	Simple operation, sensitivity and Similar to the Folin-phenol method, The reagent is very stable and anti-interference Strong ability, such as: detergent SDS, Triton-100, hydrochloric acid Guanidine and urea had no effect. Yes Different types of egg white change The heterogeneity is small	It takes longer and is vulnerable Interference from reducing substances (e.g., DTT, mercaptoethanol, etc.)

The following focuses on ultraviolet spectrophotometry.

The 20 amino acids that compose proteins do not absorb light in the visible spectrum. However, tyrosine (Tyr), phenylalanine (Phe), and tryptophan (Trp) exhibit light absorption in the near-ultraviolet region (220-300 nm) due to their aromatic ring structures containing conjugated double bonds. The maximum absorption peaks (λmax) for these amino acids are: tyrosine at 275 nm, phenylalanine at 257 nm, and tryptophan at 280 nm. The varying quantities of these amino acid residues in different proteins result in distinct absorption peaks across proteins, as illustrated in Figures A, B, and C. Additionally, solutions of the same protein concentration at 280nm exhibit different absorbance values. Preliminary statistics show that 1,800 protein and protein subunit samples at 1.0 mg/mL concentration demonstrate absorbance values ranging from 0.3 to 3.0 at 280 nm, with an average of 1.25±0.51.

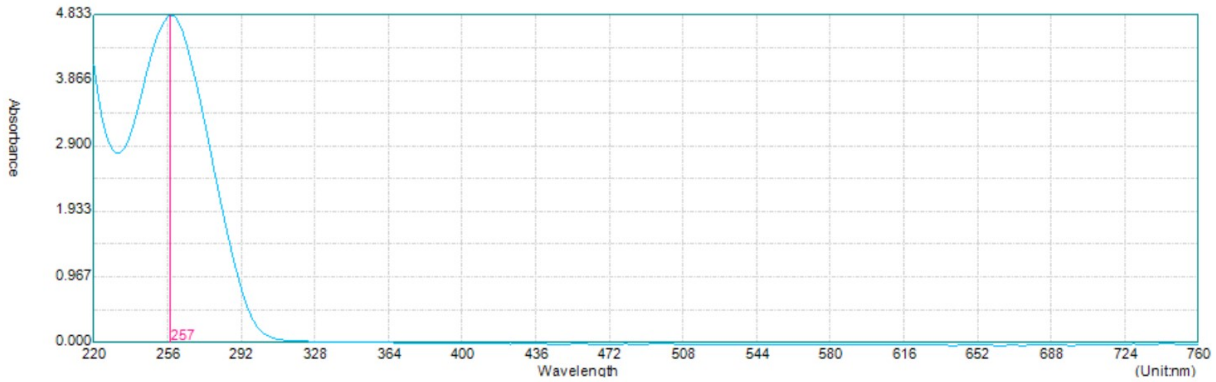


Figure A The total protein of lean pork tissue extracted with the high efficiency protein lysate extraction reagent from Baitek Biology showed an absorption peak at 257nm

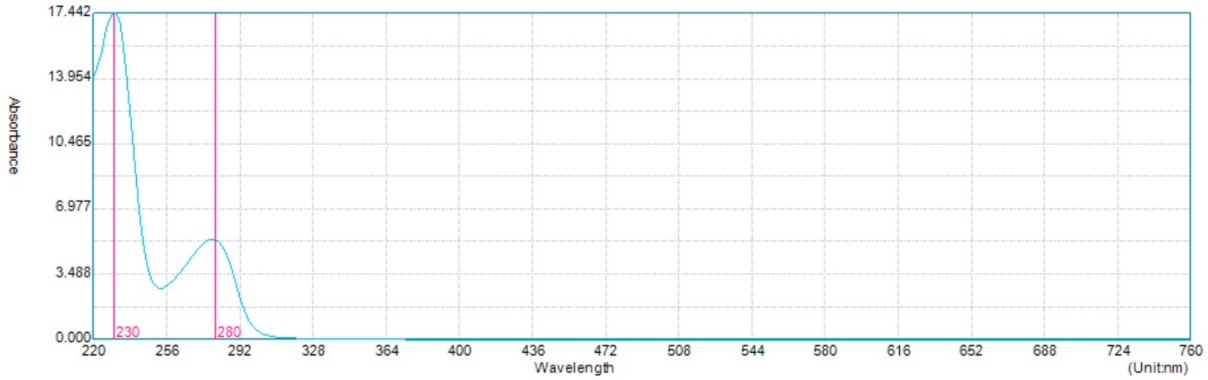


Figure B shows the absorption peak of BSA (bovine serum albumin) dissolved in pure water at 280nm (the peak at 230nm is the absorption peak of small molecules such as salts)

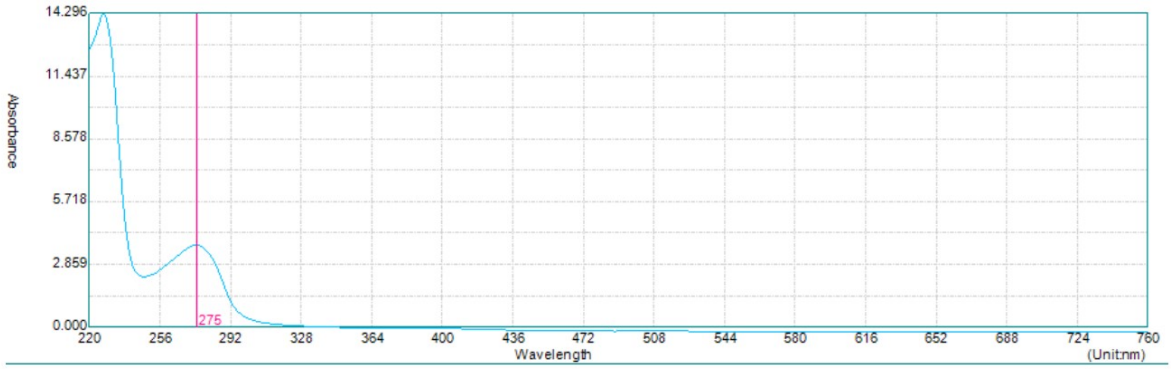


Figure C shows that the absorption peak of a certain enzyme (a protein) is at 275nm

The commonly used ultraviolet absorbance photometry methods are as follows:

1. 280nm light absorption method

See 3.2.2 Protein measurement in this manual for details.

Advantages: simple, fast, no sample consumption, low salt concentration does not interfere with the determination.

Disadvantages: a. There is a certain error with the protein whose tryptophan and tyrosine content differs greatly from that of standard protein.

b. The sample contains purine, pyrimidine and other substances that absorb ultraviolet light, which will cause great interference.

2. Absorption difference method at 280nm and 260nm

Nucleic acids exhibit strong UV absorption, with their absorbance at 280nm being 10 times stronger than that of proteins (per gram). However, nucleic acids demonstrate even stronger absorption at 260nm, where their peak absorption occurs near this wavelength. The extinction coefficient at 260nm is twice that of 280nm, whereas proteins show the opposite pattern – their UV absorbance at 280nm exceeds that at 260nm. Generally speaking:

The light absorption ratio of pure protein is A280/A260 =1.8

The ratio of light absorption of pure nucleic acid is A280/A260 =0.5

The protein solution containing nucleic acid can be measured separately at A280 and A260. The protein concentration can be calculated by the following empirical formula based on the absorption difference:

Protein concentration = 1.45×A280−0.74×A260 (mg/ml)

This empirical formula was established from data measured by a series of known mixtures of proteins (yeast enolase) and nucleic acids (yeast nucleic acid) in different concentrations.

3. Absorption difference method at 215nm and 225nm

Protein peptide bonds exhibit strong UV absorption at 200-250 nm. The intensity of this absorption is proportional to protein concentration within a specific range, with shorter wavelengths demonstrating greater absorption. Selecting 215nm minimizes interference and light scattering. By comparing the absorbance difference between 215nm and 225nm to single-wavelength measurements, errors caused by non-protein components can be reduced. Therefore, the 215 nm-225nm absorbance difference method is recommended for protein concentration determination in dilute solutions.

The following empirical estimation is commonly used: protein concentration (mg/mL) =0.144 (A215nm-A225nm) The measurement range is 20~100ug/mL protein.

Sodium chloride, ammonium sulfate, phosphoric acid, boric acid, Tris and other buffers have no interference effect. However, 0.1mol/L acetic acid, succinic acid, phthalic acid and barbital buffer have large absorption at 215nm, so they cannot be used, and must be reduced to 5mmol/L to have no significant effect

4. Peptide bond determination

The intensity of light absorption at 238nm in protein solutions is directly proportional to the number of peptide bonds. Therefore, a series of 50-500 mg/ml protein solutions with known concentrations (5.0 mL) can be prepared using standard protein solutions. The A238 absorbance at 238nm is measured, and a calibration curve is plotted with A238 as the vertical axis and protein concentration as the horizontal axis. The concentration of unknown samples can be determined using this calibration curve.

When column chromatography is performed on protein solution, the eluent can also be used to detect the protein peak at 238nm.

This method demonstrates higher sensitivity than the 280nm absorption method. However, various organic compounds such as alcohols, ketones, aldehydes, ethers, organic acids, amides, and peroxides may interfere with the results. Therefore, it is advisable to use inorganic salts, inorganic bases, or aqueous solutions for analysis. When organic solvents are present, samples should first be dried by evaporation or other methods to remove interfering substances. Afterward, dissolve them in water, dilute acid, or dilute alkali before conducting measurements.

Some special proteins have their own calculation formula, for example:

Abs=1 mg/ml: a general concentration calculation standard. At a light path of 10mm, it is assumed that a protein solution of 10mg/ml has a wave length of 280nm and an absorbance coefficient of 10.

BSA: BSA is used as the concentration calculation standard. At a light path of 10mm, the

absorbance coefficient of 0.1% (i.e., 10mg/ml) BSA solution at 280nm is 6.7.

IgA: IgA was used as the concentration calculation standard. At a light path of 10mm, the absorbance coefficient of 1% (i.e., 10mg/ml) IgA solution at 280nm was 13.7.

Lysozyme: Lysozyme was used as the concentration calculation standard. At a light path of 10mm, the 1% (i.e., 10mg/ml) Lysozyme solution had an absorbance coefficient of 26.4 at 280nm.

- Sample concentration: The unit of the concentration of the sample to be tested is mg/ml. The calculation formula of the sample concentration is as follows:

- Concentration （1Abs=1 mg/ml）=A280×10

- Concentration （BSA） =A280×10/6.7 - Concentration （BSA） =A280×10/6.7

- Concentration （IgA） =A280×10/13.7 - Concentration （IgA） =A280×10/13.7

- Concentration （Lysozyme） =A280×10/26.4

Appendix F Absorption coefficient of proteins

-------Introduction to extinction coefficient, focusing on the use of spectrometer to measure protein concentration

brief introduction

In various applications involving polypeptides or proteins, determining protein ratios or estimating the concentration of pure protein samples is crucial. Amino acids containing benzene ring structures (such as tyrosine, tryptophan, and phenylalanine) exhibit strong UV absorption. Therefore, the UV absorption capacity of proteins and peptides correlates proportionally with their aromatic amino acid content and total concentration. Once a given protein's absorbance coefficient is determined (determined by its fixed amino acid composition), the protein concentration in solution can be calculated through its absorbance.

For most proteins, UV light absorption can detect samples as low as 100 μg/mL. However, estimating protein concentrations in complex solutions like cell lysates using UV light absorption is inaccurate because the proportion of proteins with different absorption coefficients remains unknown. Moreover, proteins are not the only molecules absorbing ultraviolet light. Mixed solutions often contain interfering substances such as nucleic acids that can interfere with protein concentration measurements using UV absorption methods. Nevertheless, in laboratory settings, measuring protein concentrations in aqueous solutions at 280nm exhibits minimal interference from other compounds.

Among the 20 amino acid residues that compose proteins, only tryptophan (W) and tyrosine (Y), along with cysteine (Cys, C) which has a relatively minor impact, significantly contribute to the absorbance at 280nm for peptides or proteins. The aforementioned phenylalanine (Phe, F) exhibits absorption only at lower wavelengths (240-265 nm).

Absorbance and extinction coefficient

The ratio of the radiation power (P) of the sample to the radiation power (P0) incident on the sample is called the transmittance.

T = P/P0

Absorbance (A) is defined as the logarithm of the reciprocal of the transmittance (base 10).

$A = -\log T = \log (1/T)$

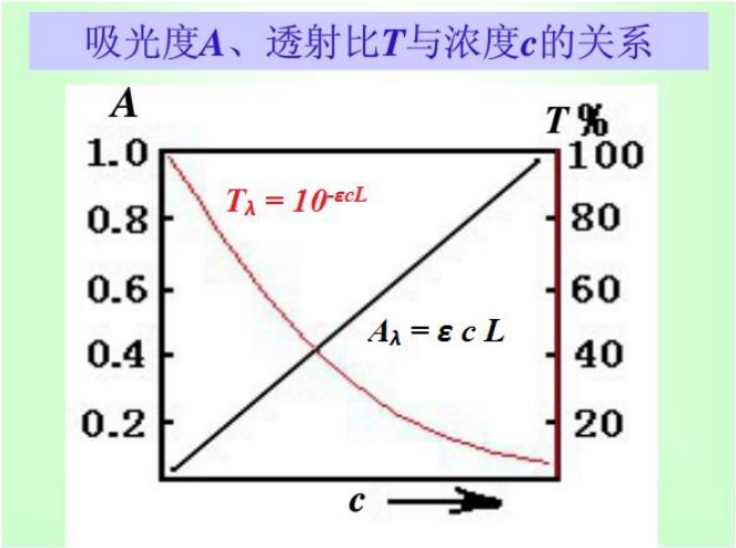
In a spectrophotometer, monochromatic plane parallel light enters the sample at an

angle perpendicular to the surface of the sample plane.

Under these conditions, the transmittance and absorption of a sample depend on molar concentration (c) (in units mol/L) and optical path length (L)

(Unit centimeters) and the molar absorption coefficient (ε) of the dissolved substance at a specified wavelength (λ).

$T\lambda = 10^{-\varepsilon cL}$ or $A\lambda = \varepsilon\ c\ L$ **$T\lambda = 10^{-\varepsilon cL}$ or $A\lambda = \varepsilon\ c\ L$**



Beer's Law states that the molar absorbance of a solute dissolved in a given solvent at a specific wavelength remains constant (absorbance is proportional to concentration). For this reason, the absorbance coefficient is referred to as the molar absorbance coefficient or molar extinction coefficient. Since transmittance and absorbance are dimensionless quantities, the unit of the molar absorbance coefficient must be expressed using concentration and path length measurement units. Consequently, the molar absorbance coefficient is expressed in units of M^-1·cm^-1. Standard laboratory spectrophotometers are equipped with 1 cm-wide sample cuvettes; therefore, the path length is generally assumed to be equal to 1 cm, and the formula is simplified in most calculations.

Aλ = ε c L = ε c (when L = 1cm)

The molar absorbance coefficient of a peptide or protein is determined by the content of its tryptophan (W), tyrosine (Y), and cysteine (C). At 280 nm, this value can be estimated by the weighted sum of the molar absorbance coefficients of these three amino acids at 280 nm, as described by the following equation:

ε = (nW×5500) + (nY×1490) + (nC×125)

Where n is the number of each residue and the weighting value is the molar absorbance coefficient of each amino acid at 280nm.

The protein concentration in the solution was determined by absorbance

Using the concentration expression of Bill's Law, it is easy to see what values are needed to determine the concentration of a peptide or protein solution:

c = A / ε L (where L = 1 cm)

The absorbance of the polypeptide or protein is measured and divided by the calculated or known molar extinction coefficient to obtain the molar concentration of the polypeptide or protein solution. Multiplying by the molecular weight gives the mass

concentration.

In this case, if the molar extinction coefficient is unknown, the amino acid composition of the peptide or protein must be known and its molar extinction coefficient calculated using the formula described in the previous section.

For complex molecules such as peptides or proteins, there is no single correct extinction coefficient value. Even minor variations in buffer type, ionic strength, and pH can at least slightly affect absorbance readings. Most protein preparations, even those of identical purity, may exhibit slight differences in morphology and degree of modification (such as oxidation) that impact absorption rates. Therefore, the optimal extinction coefficient value should be determined empirically by studying proteins dissolved in the same buffer at known concentrations as samples.

As a reference, the absorbance coefficients (i.e., extinction coefficients) of numerous proteins have been documented in biochemical and molecular biology literature. These values provide sufficient accuracy for routine laboratory applications requiring protein concentration assessment. Most reported protein extinction coefficients are measured at 280nm or near this wavelength in phosphate buffers or other physiological solutions.

1Comparison of molar extinction coefficient and absorbance of% solution

The application of molar extinction coefficient in calculation is to use molar concentration to represent concentration. The formula is as follows:

A/εmol = molar concentration

And C = M CW × molar concentration

Note: 1.C concentration, in mg/mL or g/L.

2. MW The molecular weight of a protein, in Dalton (Dalton), Da, or D.

3. Molar concentration, in units of M, also written as mol/L.

However, many sources—including the reference samples cited above—do not provide molar extinction coefficients. Instead, they offer absorbance values (A280nm) measured in 1cm cuvettes for samples at 1% concentration (g/100 mL). These values can be interpreted as percentage absorbance coefficients, with units expressed as g/100mL−1 cm−1 rather than M−1cm−1.

Therefore, when these values are used as extinction coefficients in the general formula, the unit of concentration is percentage concentration (i.e., 1% = 1g/100mL = 10mg/mL).

A/ε% = percentage concentration

If you want to use mg/mL as the unit, then a adjustment factor of 10 must be added when using these percentage absorbance coefficients. (That is, the 10 mg/ml unit must be converted to the concentration unit of 1 mg/ml).

(10 A/ε%) = concentration (unit: mg/mL)

Thus, the relationship between the molar extinction coefficient (εmolar) and the percentage absorption coefficient (εpercent) is as follows:

(εmol) 10 = (ε%) × (protein molecular weight MW)

Additionally, absorbance values for 0.1% (mg/mL) protein solutions from other sources are provided, as this measurement unit is more convenient and commonly used in protein work compared to the percentage absorbance coefficient. Variations in such reports require careful reading of specified values.

To ensure the understanding and correct application of units of measurement.

example：

A. Protein and protein mixtures with unknown extinction coefficients

When the extinction coefficient of a protein or its mixture is unknown and there are no other interfering substances, a rough estimation of protein concentration requires a solution. A common assumption is that εpercent = 10. Most proteins have an extinction coefficient (εpercent) ranging from 4.0 to 24.0. Therefore, even if any given protein's extinction coefficient deviates significantly from εpercent = 10, the average εpercent for a mixture of many different proteins may still be around 10.

B. Antibodies (immunoglobulins)

The extinction coefficients (ε%) of most mammalian antibody proteins (i.e., immunoglobulins) typically range between 12 and 15. For 2 standard m antibody mn280280msolutions, we assume A80n=14 or An=Al=1.4. For a typical IgG with molecular weight (MW)=150,000, this corresponds to a molar extinction coefficient (εmolar) equal to 210,000 $M^{-1} \cdot cm^{-1}$.

C. Bovine serum albumin (BSA)

4. The mg/mL bovine serum albumin (BSA) was dissolved in a 0.9%NaCl solution. Numerous data on the absorption rate of BSA have been reported in the literature, but the absorbance of 1% solution at 280nm is generally around 6.6. The calculation is as follows:

εpercent c L / 10 = A

[(6.6)(2.000)(1)] / 10 = 1.320

The 2mg/mL bovine serum albumin (BSA) was dissolved in 0.9%NaCl solution and the absorbance at 280nm was 1.320.

Assuming that a standard albumin has an absorbance reading of 1.346 relative to water at 280 nm, and assuming the specified percentage absorption value, the calculated concentration is as follows:

(A / εpercent) × 10 = c mg/ml

(1.346 / 6.6) × 10 = 2.039mg/mL

Assuming MW =66400, the molar extinction coefficient of BSA at 280nm is about 43,824M-1cm-1.