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Meningococcal Group W135 Total Sugar Quantitative Detection Kit Manual

Cat#17-0137

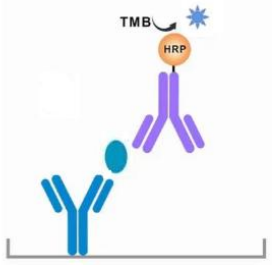
This kit is used for the detection of total sugar of Meningococcal Group W135 in samples.

research use only and not for diagnostic purposes.

Table of Contents

1．Experimental Principle

This product uses the principle of a sandwich ELISA with a monoclonal antibody against Meningococcal Group W135 polysaccharide coated on the ELISA plate. After the addition of the sample and reaction, unbound materials are washed away. Another HRP-labeled monoclonal antibody against Meningococcal Group W135 polysaccharide is added, forming a complex of coated antibody-antigen-antibody-HRP. The degree of TMB color development indicates the content of total sugar of Meningococcal Group W135 in the sample.



2．Instructions for Use

Please read this manual carefully before starting the experiment.
- Follow good laboratory practices: Wear gloves, lab coats, and safety goggles at all times. Do not eat, drink, or smoke in the laboratory area.
- All biological materials should be treated as potentially hazardous and disposed of according to established safety procedures.
- Do not mix or substitute reagents or materials from other kit batches or suppliers; performance cannot be guaranteed if substituted.

3．Materials and Reagents Provided

Components of the kit	Quantity	Storage Conditions
Pre-coated plate	8 wells × 12 strips	2~8℃
Detection antibody (500×)	50μL × 1 tube	-15 ~ -25℃
Chromogen Solution A	7mL × 1 bottle	2~8℃
Chromogen Solution B	7mL × 1 bottle	2~8℃
Termination solution	7mL × 1 bottle	2~8℃
BSA	3g/bag × 2 bags	2~8℃
Detection antibody diluent	12mL × 1 bottle	2~8℃
20×PBST	50mL × 1 bottle	2~8℃
Sealing membrane	2 sheets	2~8°C/Room temperature
Manual	1 copy	2~8°C/Room temperature

4．Materials Required for the Experiment but Not Provide

These materials are not included in the kit but are needed for the test:
- Reference standards

- Microplate reader, deionized water, multi-channel and single-channel pipettes, dilution tubes, shaker incubator.

5．Storage Conditions and Shelf Life

- Detection antibody (500×), store at -15 ~ -25°C, other components at 2~8°C away from light.
- Validity period of 12 months.

6．Operational Tips

- Avoid creating bubbles when preparing solutions or adding samples.
- Replace tips promptly to avoid cross-contamination of samples or reagents.
- Ensure the ELISA plate is covered with a sealing membrane during incubation.
- All samples should be thoroughly and gently mixed.
- Incubate the ELISA plate on a shaker incubator during all incubation steps.

7．Reagent Preparation

- Equilibration: Bring the required reagents to room temperature (18 ~ 25°C) and equilibrate for 30 minutes.
- 20×PBST may contain precipitates, which is normal. If the precipitate does not dissolve by gentle stirring, it can be heated in a 37°C oven to dissolve the precipitate before use.

7.1 Preparation of 1×PBST

Take 1 bottle of 20×PBST and dilute with deionized water to 1000mL, mix well and set aside for use. The amount of solution can be adjusted according to the experimental needs, prepare as needed.

7.2 Preparation of 5% BSA diluent

Accurately weigh 5.0g of BSA and completely dissolve it in 100mL of 1×PBST, mix well and set aside for use as 5% BSA diluent.

The amount of solution can be adjusted according to the experimental needs, prepare as needed.

7.3 Preparation of enzyme conjugate-detection antibody working solution

Calculate the volume of working solution needed for the experiment, take an appropriate amount of enzyme conjugate (500×) and dilute with detection antibody diluent 100 times, then further dilute 5 times, mix well and set aside for use.

8．Reference and Sample Preparation

-Prepare different concentrations of reference standards (2-fold gradient dilution) with 1% BSA diluent: 1280ng/mL, 640ng/mL, 320ng/mL, 160ng/mL, 80ng/mL, 40ng/mL, 20ng/mL, 10ng/mL, 5ng/mL, 2.5ng/mL, 1.25ng/mL, 0.625ng/mL, 0.3125ng/mL, 0ng/mL (1% BSA diluent).

- Dilute the test samples with 5% BSA diluent to the linear range of the standard curve for detection. If the concentration of the test sample is unknown, it can be diluted 10 times, 30 times, 100 times, 300 times, and 1000 times, and then tested to select the OD value within the standard curve range to back-calculate the sample concentration (ng/mL). Finally, the sample concentration should be multiplied by the sample dilution factor to obtain the measured value.

- Prepare new reference and sample solutions each time, and discard used reference and sample solutions.

9．Flowchart

9.1 Prepare all reagents, samples, and reference standards according to the manual.
9.2 Add reference standards or samples (100μL) into the wells of the microplate, incubate at 37°C with shaking for 60 minutes.
9.3 Wash with 1x PBST three times.
9.4 Add the enzyme-labeled reagent (100μL), and incubate at 37°C for 30 minutes.
9.5 Wash with 1x PBST three times.
9.6 Add solution A and B 50μL, and let it react at room temperature in the dark for 5 minutes.
9.7 Add 50μL of the stopping solution to all wells, and read the OD values.

10．Operating Procedures

- Prepare all reagents, references, and samples according to the requirements of the previous sections.
- The ELISA plate is ready to use at any time, and it is not necessary to rinse before use.

10.1 Sample Addition

Add all references and samples to the ELISA plate, mark them, add 100μL of sample per well (set up duplicate wells), seal the plate with a sealing membrane, and place it in a 37°C constant temperature shaker incubator, 300rpm, incubate for 60 minutes.

10.2 Washing

Discard the liquid in each well, add 350μL of 1×PBST to each well, let it stand for 30 seconds, discard the liquid, and repeat 3 times. After the last wash, gently blot dry on a paper towel.

10.3 Addition of Enzyme Conjugate

Add 100μL of enzyme conjugate working solution to each well, seal the plate with a new sealing membrane, and place it in a 37°C constant temperature shaker incubator, 300rpm, incubate for 30 minutes.

10.4 Washing

Repeat step 10.2.

10.5 Color Development

Add 50μL of Substrate Chromogen Solution A to each well, then add 50μL of Substrate Chromogen Solution B to each well, gently mix and incubate in the dark at room temperature (25°C±4°C) for 5 minutes.

10.6 Termination

Add 50μL of termination solution to each well, gently mix.

10.7 Reading

Select the main wavelength of the microplate reader at 450nm, reference wavelength at 630nm, and measure the absorbance value (OD value=OD450-OD630) of each well.

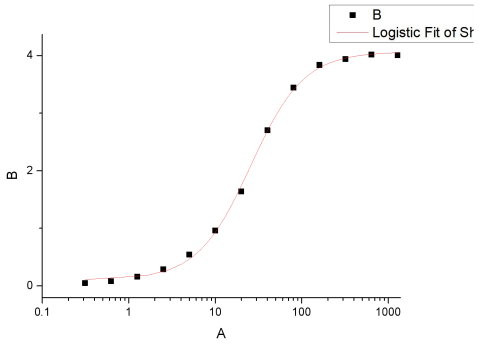
11．Result Processing

11.1 Standard Curve

Calculate the average OD value of the reference (see example below, for illustration only, specific to actual measurement):

Standard Curve ng/mL	OD1	OD2	Average OD
4000	3.940	4.069	4.005
2000	4.039	3.990	4.015
1000	3.885	3.996	3.941
500	3.911	3.759	3.835
250	3.519	3.367	3.443
125	2.776	2.626	2.701
62.5	1.666	1.614	1.640
31.25	0.976	0.950	0.963
15.625	0.542	0.546	0.544
7.8125	0.286	0.285	0.286
3.90625	0.160	0.162	0.161
NC	0.082	0.083	0.083

Fit the standard curve concentration and corresponding average OD values using a four-parameter fitting method to obtain the standard curve.



Four-parameter fitting standard curve (linear correlation coefficient R^2=0.99881)

11.2 Calculation of Sample Concentration

Select the OD value within the standard curve range to back-calculate the sample concentration, which is the measured value (ng/mL). Multiply the measured value by the sample dilution factor to obtain the sample concentration.

12. Product Performance Indicators

- Linear range: 1.25 ~ 160ng/mL
- Sensitivity: <1.25ng/mL.
- Specificity: No cross-reactivity with other A, C, Y group meningococcal polysaccharides, good specificity.

13. Problems and Solutions

Problem	Cause	Solution
Poor standard curve linearity	Improper dilution of the standard curve	Check the pipette, use a calibrated pipette, standardize operations, and redilute.
Low signal value	Insufficient incubation time	Ensure adequate incubation time。
	Insufficient reagent amount or improper dilution	Check the pipette, ensure correct and sufficient reagent preparation

	Prolonged exposure of color development solution	The color development solution should be stored away from light, replace with a new color development solution.
High CV value	Inadequate washing	Follow the washing steps strictly.
	Contaminated washing solution	Replace with a new washing solution.
Insufficient sensitivity	Improper storage of the ELISA kit	Store according to the manual instructions
Precipitation in the diluent	Precipitation and/or coagulation of components in the diluent	Heat the diluent to 37ºC to completely dissolve the precipitate.