M5 HiPer Chromogenic LAL Endotoxin Assay Kit
内毒素定量检测试剂盒使用说明书

产品名称	单位	货号
M5 HiPer Chromogenic LAL Endotoxin Assay Kit	16T	MF835-01

【储存条件】

The kit should be stored dry at room temperature for up to one month. For longer storage, the kit can be kept at 2–8°C for up to 24 months. Do not freeze the kit or any of its components.

【产品简介】

M5 HiPer Chromogenic LAL Endotoxin Assay Kit is designed to be a quantitative In Vitro end-point endotoxin test for human and animal parenteral drugs, biological products, and medical devices. This method utilizes a modified Limulus Amebocyte Lysate and a synthetic color producing substrate to detect endotoxin chromogenically in a broad range of 0.01 - 1 EU/ml. In addition, any sample with color (e.g. cell bacterial culture medium, serum or blood etc.) can not be assayed by this kit.

【产品特点】

Highly Sensitive: Detect endotoxin concentration in the range of 0.01 - 1 EU/ml
Fast: The incubation period can be shortened to 14 minutes
Reliable: Color-stabilizer ensures accurate results
Ready-to-use: Kit includes endotoxin-free tips and tubes, LAL reagent water and incubation rack
Broad application: Quantitative in vitro end-point endotoxin test

【产品组成】

LAL Reagent Water, 50 ml/bottle	1 bottle
Limulus Amebocyte Lysate (LAL)	1 Vial
E. coli Endotoxin Standard	1 Vial
Chromogenic Substrate	1 Vial
Buffer S for Color-stabilizer #1	10 ml
Color-stabilizer #1	1 Vial
Color-stabilizer #2	1 Vial
Color-stabilizer #3	1 Vial
Endotoxin-free tubes	25
Endotoxin-free Tips, 200 µl	1 box (96 tips)
Endotoxin-free Tips, 1000 µl	2 bags (12 tips)
Incubation Rack	1

【客户自备】

1. Sodium hydroxide, 0.1 N, dissolved in LAL Reagent Water, for pH adjustment.
2. Hydrochloric acid, 0.1 N, diluted in LAL Reagent Water, for pH adjustment.
3. Water bath or heating block set at 37°C ± 1.0°C.
4. Spectrometer or filter photometer with a 545 nm filter.
5. Vortexer.
6. Timer.

【操作步骤】

1. Specimen Preparation

All materials or diluents used for specimen collection and test reagent preparation must be endotoxin-free. Use aseptic technique at all times. Samples to be tested must be stored in such a way that all bacteriological activity is stopped or the endotoxin level may increase over time. For example, samples canbe stored at 2-8 ℃ within 24 hours before use, but need to be stored frozen if not used within 24 hours.

Dissolve or dilute test specimen using LAL Reagent Water. Since the LAL-endotoxin reaction is pH dependent, the pH value of the mixture should be between 6.0 and 8.0 to ensure standard and data linearity. Adjust the pH of the specimen with endotoxin-free 0.1N sodium hydroxide or 0.1N hydrochloric acid. Always measure the pH of an aliquot of the bulk sample, take care to avoid contamination by the pH electrode used. If the specimen contains interfering substances, dilute the specimen until that the interference is eliminated.

2. Reagent preparation

Limulus Amebocyte Lysate (LAL)
Reconstitute lyophilized lysate by adding 1.7 ml LAL Reagent Water. Swirl each reconstitution gently for 30seconds, avoid foaming. Reconstituted lysate remains stable if stored at -20°C for one week. For long-term usage, freeze the lysate at -80°C immediately after reconstitution. Avoid repeated freeze and thaw cycles.

Chromogenic Substrate
Reconstitute the substrate by adding 1.7 ml of LAL Reagent Water to a final concentration of ~2 mM. Once reconstituted, the substrate solution is stable for one month when stored at 2°C - 8°C.

PROTECT SUBSTRATE FROM LONG-TERM EXPOSURE TO LIGHT

Stop Solution
Reconstitute the Color-stabilizer #1 (Stop Solution) with 10 ml of buffer S. The reconstituted Stop Solutionis stable for one week when stored at 2°C - 8°C.

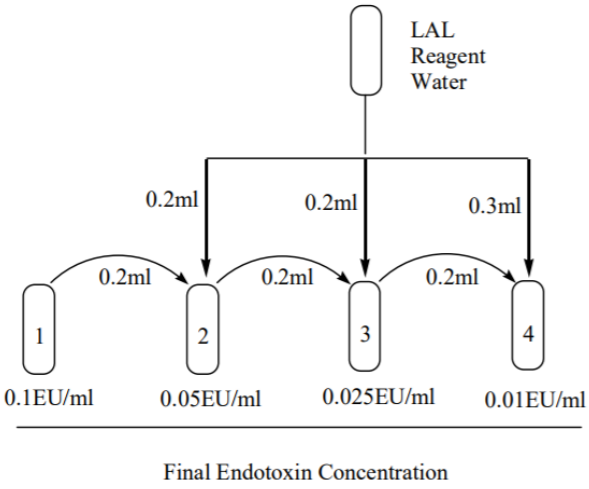
Color-stabilizer #2 and #3
Reconstitute Color-stabilizer #2 and #3 by adding 10 ml of LAL Reagent Water for both, respectively. Eachreconstitution is stable for one week when stored at 2°C - 8°C.

Endotoxin Standard Solution
The amount of lyophilized endotoxin standard supplied in this kit should be referred to the label on the vial of the endotoxin standard in the kit. Dissolve lyophilized endotoxin standard by adding 2 ml of LAL Reagent Water. Mix thoroughly for 15 minutes with a vortexer to obtain an endotoxin stock solution. The reconstituted endotoxin stock solution is stable for one week when stored at 2-8°C. DO NOT FREEZE THE

ENDOTOXIN STOCK SOLUTION.
Prepare 1EU/ml endotoxin solution before making standard serial dilutions. For example, if the endotoxinstock solution is 5 EU/ml, dilute 0.2 ml of 5 EU/ml endotoxin stock solution with 0.8 ml of LAL Reagent

Water to make the 1 EU/ml solution.
In each assay, at least four endotoxin standard solutions covering desired concentration range should be prepared to generate a standard curve. If the endotoxin concentration for the test sample is expected to be in the range of 0.01 - 0.1 EU/ml, the serial endotoxin standard solutions could be 0.1, 0.05, 0.025 and 0.01EU/ml, respectively. If the endotoxin concentration in sample is expected in the range of 0.1 - 1 EU/ml, the serial endotoxin standard solutions could be 1, 0.5, 0.25 and 0.1 EU/ml, respectively.

An example of the preparation of serial endotoxin standard solutions is outlined in the figure below. Each solution should be mixed thoroughly for 30 seconds with a vortexer.



3. Test Procedure

1) Carefully dispense 100 µl of standard or test sample into endotoxin-free vials. All samples should be mixed thoroughly for 30 seconds with a vortexer. Avoid foaming/bubbles.

Each test must include a blank as well as at least four endotoxin standards in duplicate. The blank sample vial contains 100 µl of LAL Reagent Water instead of test sample.

2) Add 100 µl of reconstituted LAL to each vial. Cap the vials and mix well by swirling gently.

3) If the endotoxin concentration in sample is expected in the range of 0.01 - 0.1 EU/ml, incubate the rack with all vials at 37°C±1°C for T1 using a water bath or heating block. If the endotoxin concentration is expected in the range of 0.1 - 1 EU/ml, incubate at 37°C±1°C for T2.

Note: The optimal value of T1 and T2 should be referred to the label on the kit.

4) After proper incubation, add 100 µl of reconstituted chromogenic substrate solution to each vial. Cap the vials and swirl gently to mix well. Do not shake or vortex to avoid foaming. Incubate at 37°C±1°C for 6 minutes.

5) Add 500 µl of reconstituted Color-stabilizer #1 (Stop Solution) to each vial and swirl gently to mix well.

Do not shake or vortex to avoid foaming. Add 500 µl of reconstituted Color-stabilizer #2 to each vial and mix well. Finally add 500 µl of reconstituted Color-stabilizer #3 to each vial. Gently swirl each vial to mix well. Bubbles must be avoided.

6) Read the absorbance of each reaction vial at 545 nm using distilled water as blank to adjust the photometer to zero absorbance. The full testing procedure is summarized in the table below.

The full testing procedure is summarized in the table below.

	Sample	Blank
Test sample or standard (ml)	0.1	
LAL Reagent Water (ml)		0.1
LAL (ml)	0.1	0.1
Mix well and incubate at 37°C±1.0°C (min)*	T1 or T2	T1 or T2
Substrate Solution (ml)	0.1	0.1
Mix well and incubate at 37°C±1.0°C (min)	6	6
Stop Solution (ml)	0.5	0.5
Color-stabilizer #2 (ml)	0.5	0.5
Color-stabilizer #3 (ml)	0.5	0.5
Mix well and read the absorbance at 545 nm		

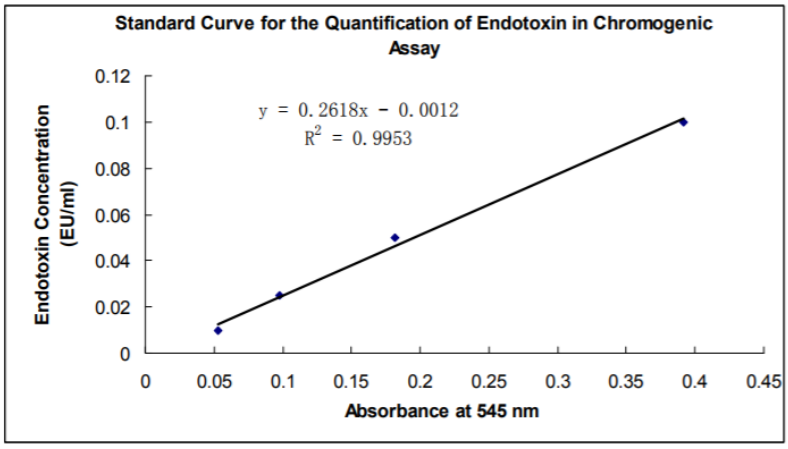
*Incubation time in this step is depending on the expected endotoxin level in test samples. If the endotoxin concentration in sample is expected in the range of 0.01 - 0.1 EU/ml, incubate the rack with all vials at 37°C±1°C for T1. If the endotoxin concentration is expected in the range of 0.1 - 1 EU/ml, incubate at 37°C±1°C for T2.

4. CALCULATION OF ENDOTOXIN CONCENTRATION

Under the standard conditions, the absorbance at 545 nm shows a linear relationship with the concentration in the range of 0.01 to 1 EU/ml. Plot the absorbance for the four standards on the x-axis and the corresponding endotoxin concentration in EU/ml on the y-axis. Draw a best-fit straight line between these points and determine endotoxin concentrations of samples graphically.

Example Data

Vial No.	Sample	Absorbance at 545nm	Δ Absorbance
1	LAL reagent water (Blank)	0.035	—
2	0.01 EU/ml Standard	0.088	0.053
3	0.025 EU/ml Standard	0.133	0.098
4	0.05 EU/ml Standard	0.217	0.182
5	0.1 EU/ml Standard	0.427	0.392



If the mean absorbance of a sample is x, the endotoxin concentration of the sample will be (0.2618x – 0.0012) EU/ml. All incubations were performed for 45 min. The figure above shows an example standard curve, the absorption values of standards may be different in different assays.

Note: The dilution of standards and incubation temperature would be critical factors that can influence the absorption value, so it's important to make sure that the endotoxin standard is fully dissolved, and the incubation temperature should strictly be kept at 37°C±1°C.

PERFORMANCE CHARACTERISTICS

Linearity

The linearity of the standard curve within the concentration range used to predict endotoxin values need to be verified for each new lot of reagents. At least 4 endotoxin standards spanning the expected concentration range should be assayed along with a blank, in duplicate. The coefficient of correlation (r) for the individual mean absorbance of the standards vs. their corresponding endotoxin concentration should be ⩾0.980.

Reproducibility

Replicate samples should be run in order to establish good technique and low coefficient of variation. The coefficient of variation (C.V.) equals 100 times the standard deviation of a group of values divided by the mean and is expressed as a percent. The C.V. absorbance should be less than 10%.

【常见问题】

Problems	Possible Causes	Recommended Solutions
Atypical linearity	The reconstituted Endotoxin Standards are not completely dissolved.	Endotoxin tends to adhere to the surface of glass. Dissolve the lyophilized endotoxin standard with 2 ml LAL Reagent Water as described in the protocol and mix endotoxin standard dilutions for 15 minutes by vortexing.
	The sample pH value is not suitable for the assay.	Adjust sample pH value to 6 – 8 as described in the protocol
The blank shows a higher OD than endotoxin standard.	The materials (e.g. tips, vials etc.) contacting with the specimen or test reagents may be contaminated.	Perform the assay in a laminar flow cabinet at room temperature. Wear disposable gloves and use endotoxin-free materials in order to avoid contamination.

【备注】
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