Version 05-05/24

User's Manual

LPS (Lipopolysaccharides) ELISA Kit

REF **DEIA-LL289**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

For quantitative detection of LPS in bacterial cell walls of gramnegative bacteria or in tissue homogenates and other biological fluids.

Principles of Testing

This kit was based on Competitive-ELISA detection method. The microtiter plate provided in this kit has been pre-coated with anti-LPS antibody. During the reaction, LPS in the sample or standard competes with a fixed amount of Biotin- Antigen. Excess conjugate and unbound sample or standard are washed from the plate. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. Then TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of LPS in the samples is then determined by comparing the OD of the samples to the standard curve.

Reagents And Materials Provided

No.	Item	Specifications(96T)	Storage
E001	ELISA Microplate(Dismountable)	8×12	2-8°C/-20°C
E002	Lyophilized Standard	2vial	2-8°C/-20°C
E039	Sample Dilution Buffer	20ml	2-8°C
E003	Biotin-labeled Antigen(Lyophilized)	1vial	2-8°C(Avoid Direct Light)
E005	10mM PBS	200ul	2-8°C
E040	Antigen Dilution Buffer	10ml	2-8°C
E034	HRP-Streptavidin Conjugate(SABC)	120ul	2-8°C(Avoid Direct Light)
E049	SABC Dilution Buffer	10ml	2-8°C
E024	TMB Substrate	10ml	2-8°C(Avoid Direct Light)
E026	Stop Solution	10ml	2-8°C
E038	Wash Buffer(25X)	15ml/30ml	2-8°C
E006	Plate Sealer	3/5pieces	
E007	Product Description	1 copy	

Materials Required But Not Supplied

1. Microplate reader (wavelength:450nm)
2. 37°C incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes

6. Deionized or distilled water

Storage

2-8°C for 6 months. (The shelf life is 6 months when unopened. After the biotin-labeled antigen is dissolved, please divide it into several small packages and freeze them at -20 °C. The biotin-labeled antigen removed from -20 °C should be stored at 2-8°C and used up within 2 weeks.)

Specimen Collection And Preparation

Tissue Homogenates:
As hemolysis blood has relation to assay result, it is necessary to remove residual blood by washing tissue with pre-cooling PBS buffer (0.01M, pH=7.4). Mince tissue after weighing it and get it homogenized in PBS (the volume depends on the weight of the tissue. Normal, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000×g to get the supernatant. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3mg.

Cell Culture Supernatant:
Centrifuge supernatant for 20 minutes at 1000×g at 2-8°C to remove insoluble impurity and cell debris. Collect the clear supernatant and carry out the assay immediately.

Cell Culture Lysate:
Commercial RIPA kits are recommended to follow the instructions provided. Generally, 0.5ml RIPA lysis buffer would be appropriate to $2x10^6$ cells, DNA must to be removed. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3mg.

Escherichia coli

1 gram of E. coli was mixed with 1ml PBS and then broken by ultra sound, the samples were centrifuged for 20 minutes at 10000rpm at 2-8°C. Collect supernatant and carry out the assay immediately.

Other Biological Fluids:
Centrifuge samples for 20 minutes at 1000×g at 2-8°C. Collect supernatant and carry out the assay immediately.

Note:
Samples to be used within 5 days can be stored at 2-8°C, besides that, samples must be stored at -20°C (assay ≤1 month) or -80°C(assay≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles. The hemolytic samples are not suitable for this assay.

Sample Dilution:

The user should estimate the concentration of target protein in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided dilution buffer, and several trials may be necessary. The test sample must be well mixed with the dilution buffer. And also standard curves and sample should be making in pre-experiment. If samples with very high concentrations, dilute samples with PBS first and then dilute the samples with Sample Dilution.

Reagent Preparation

Bring all reagents and samples to room temperature for 20 minutes before use.

Wash Buffer:

If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely been dissolved. The solution should be cooled to room temperature before use.

Dilute 30ml Concentrated Wash Buffer into 750ml Wash Buffer with deionized or distilled water. Put unused solution back at 2-8°C.

Preparation of Biotin- labeled Antigen

After the biotin-labeled antigen is dissolved, please divide it into several small packages and freeze them at -20 °C. The biotinlabeled antigen removed from -20 °C should be stored at 2-8°C and used up within 2 weeks.

Dissolve: Add 100ul 10mM PBS into tube and mix them thoroughly, divide it into several small packages and store the remaining reagent at -20 ° C.

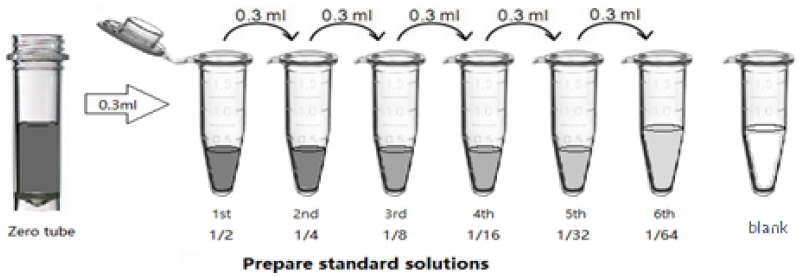
Calculate required total volume of the working solution: 55µl /well × quantity of wells. (Allow 0.1-0.2ml more than the total volume.

Preparation of Biotin-labeled Antigen Working Solution: According to the volume required for this experiment, dilute the Biotin-labeled Antigen with Antigen Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1µl of Biotin- labeled Antigen into 99µl of Antigen Dilution Buffer.)

Standards:

Add 0.5 ml Sample Dilution Buffer into one Standard tube (labeled as zero tube), keep the tube at room temperature for 10 minutes and mix them thoroughly.

Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3ml of the Sample Dilution Buffer into each tube. Add 0.3ml of the above Standard solution (from zero tube) into 1st tube and mix them thoroughly. Transfer 0.3ml from 1st tube to 2nd tube and mix them thoroughly. Transfer 0.3ml from 2nd tube to 3rd tube and mix them thoroughly, and so on. Sample Dilution Buffer was used for the blank control.



Note: It is best to use Standard Solutions within 2 hours.

Standard/Sample/Biotin-Antigen compound:

Mix 55ul diluted standards/samples with 55ul Biotin- labeled Antigen Working Solution in EP tube, mix them thoroughly.

Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution:

Prepare it within 30 minutes before experiment.

Calculate required total volume of the working solution: 0.1ml/well × quantity of wells. (Allow 0.1-0.2ml more than the total volume.)

Dilute the SABC with SABC Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1ul of SABC into 99ul of SABC Dilution Buffer.)

Assay Procedure

Washing Notes

Manual: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350ul wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material.

Automatic: Aspirate all wells, and then wash plate with 350ul wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer shall be set for soaking 1 minute. (Note: set the height of the needles; be sure the fluid can be sipped up completely).

Procedure

When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37°C. It is recommended to plot a standard curve for each test.

1. Set standard and test sample wells on the pre-coated plate respectively, and then, record their positions. It is recommended to measure each standard and sample in duplicate. **Wash plate 2 times before adding standard, sample and control (blank) wells!**
2. Add 100µl **Standard/Sample/Biotin-Antigen compound** into each well and incubate for 45 minutes at 37°C. (Solutions are added to the bottom of microplate well, avoiding inside wall touching and foaming as much as you can.)
3. **Wash:** Remove the cover, and wash plate 3 times with Wash Buffer, and let the wash buffer stay in the wells for 1 minute each time.
4. **HRP-Streptavidin Conjugate (SABC):** Add 100µl of SABC Working Solution into each well, cover the plate and incubate at 37°C for 30 minutes.
5. **Wash:** Remove the cover and wash plate 5 times with Wash Buffer, and let the wash buffer stay in the wells for 1 -2 minutes each time.
6. **TMB Substrate:** Add 90ul TMB Substrate into each well, cover the plate and incubate at 37°C in dark within 10-20 minutes. (Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells.)
7. **Stop:** Add 50ul Stop Solution into each well. The color will turn yellow immediately. The adding order of Stop Solution should be as the same as the TMB Substrate Solution.
8. **OD Measurement:** Read the O.D. absorbance at 450nm in Microplate Reader immediately after adding the stop solution.

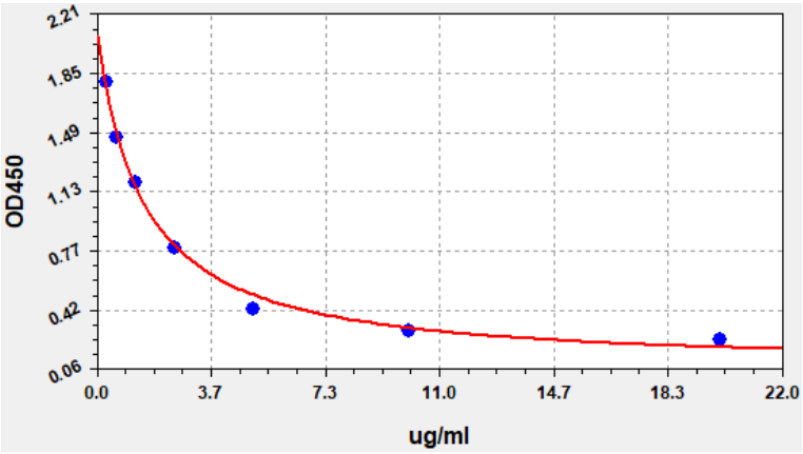
Calculation

Regarding calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of blank well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve. It is recommended to use some professional software to do this calculation, such as Curve Expert 1.3 or 1.4.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

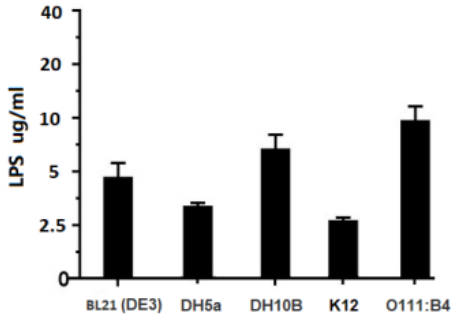
Typical Standard Curve

STD.(ug/ml)	OD-1	OD-2	Average
0	2.243	2.217	2.230
0.312	1.818	1.78	1.799
0.625	1.425	1.497	1.461
1.25	1.221	1.163	1.192
2.5	0.78	0.802	0.791
5	0.416	0.438	0.427
10	0.297	0.289	0.293
20	0.242	0.232	0.237



Reference Values

1 gram of E. coli was mixed with 1ml PBS and then broken by ultra sound, the samples were centrifuged for 20 minutes at 10000rpm at 2-8°C. Collect supernatant and carry out the assay immediately.



Precision

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Detection Range

0.313-20ug/ml

Sensitivity

0.188ug/ml

Specificity

This assay has high sensitivity and excellent specificity for detection of LPS. No significant cross-reactivity or interference between LPS and analogues was observed.

Note: Limited by current skills and knowledge, it is difficult for us to complete the cross-reactivity detection between LPS and all the analogues, therefore, cross reaction may still exist.

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of LPS and their serial dilutions. The results were demonstrated by percentage of calculated concentration to the expectation.

Sample	1:2	1:4	1:8
bacteria culture medium (n=5)	85-103%	88-94%	86-104%

Recovery

Matrices listed below were spiked with certain level of LPS and the recovery rates were calculated by comparing the measured value to the expected amount of LPS in samples.

Matrix	Recovery Range (%)	Average (%)
bacteria culture medium(n=5)	85-101	97

Precautions

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive and high background.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don't let microplate dry at the assay, for dry plate will inactivate active components on plate.
8. Don't reuse tips and tubes to avoid cross contamination.
9. Avoid using the reagents from different batches together.