General LPS ELISA Kit

Cat #: orb440611 (manual)

Size: 96 tests

For research use only, not for clinical diagnosis.

This kit is used for the quantitative determination of lipopolysaccharide (LPS) content in samples such as serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, and other biological fluids.

Detection Principle

This kit utilizes the competitive ELISA principle. Purified lipopolysaccharide (LPS) antibody is pre-coated onto a microplate. Standard solutions and test samples are sequentially added to the coated wells, along with biotin-labeled antigen. The test antigen and the biotin-labeled antigen compete for binding to the specific antibody. After incubation, unbound materials are removed by washing, and then horseradish peroxidase (HRP)-labeled avidin is added. Biotin and avidin form a high-affinity non-covalent bond. Following thorough washing, the substrate TMB is added for color development. TMB is converted to blue under the catalytic action of HRP enzyme, and finally turns yellow upon the addition of an acid solution. The higher the concentration of the test sample, the more the binding between the labeled antigen and the antibody is inhibited, resulting in a lighter color. The depth of color is positively correlated with the amount of enzyme and negatively correlated with the concentration of the test sample.

Product Composition

Reagents	Specifications（96T）	Storage Conditions
Antibody-Coated Slats	8×12	2-8℃
Standard	2 tubes	-20℃
S1 Standard/Sample Dilution Buffer	45 ml×1 bottle	-20℃
Detection Solution A	70μl×1 tube	-20℃
Detection Solution B	120μl×1 tube	-20℃
Washing Buffer (Concentrated 30×)	20ml×1 bottle	2-8℃
TMB Substrate (Avoid direct light)	9ml×1 bottle	2-8℃
Stop Solution	6ml×1 bottle	2-8℃
Plate Sealer	4 pieces	

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Manual	1 copy	

Required Instruments and Reagents

1. Microplate reader (wavelength: 450nm)
2. Precision single (0.5-10μL, 2-20μL, 20-200μL, 200-1000μL) and multi-channel pipette with disposable tips (calibration is required before use).
3. Automated plate washer
4. 37°C incubator
5. Deionized or distilled water
6. Coordinate paper
7. Measuring cylinder

Precautions

1. The kit is stored in 4℃&-20℃, the dissolved but unused standard is recommended for disposal. Do not mix kit components from different sources or batch numbers, use this product within the expiration date.
2. When the concentrated washing solution is taken out at low temperature, there may be crystal precipitation, and it can be dissolved by heating in the water bath before dilution, which does not affect the use.
3. Pipettes should be calibrated before and used for each step and to avoid errors. It is recommended to control the sampling time within 5 minutes. If there are a large number of samples, it is recommended to use a multi-channel pipette for sampling.
4. Please make a standard curve at the same time of each determination, and it is better to make a double-check well. If the content of the substance to be tested in the sample is higher than the upper limit of the reagent kit (the OD value of the sample is greater than the OD value of the first well of the standard well), please dilute a certain multiple with the sample dilution buffer before determination. The total dilution multiple shall be multiplied during calculation.
5. To avoid cross contamination, remember to replace the tip when adding different concentrations of standard, different samples and different reagents. The plate sealer is only for single use.
6. The TMB substrate should be kept colorless before adding. Do not use the TMB substrate when it turned blue.
7. Strictly follow the manual, and the test results must be based on the microplate reader reading.

Sample Collection and Storage

1. **Serum**: Blood coagulated naturally at room temperature for 60~120 min and centrifuged for 20 min (1000g). Collect supernatant carefully. If precipitation occurs during storage, centrifuge again to avoid repeated freezing and thawing.
2. **Plasma**: Select EDTA or citric acid as anticoagulant according to sample requirements and centrifuge for about 20 min (1000g). Collect the supernatant carefully and centrifuge again if precipitation occurs during storage.
3. **Cell supernatant and Other biological fluids**: When testing secreted components, collect using sterile tubes, centrifuge at 1000g for about 20 minutes, and collect the supernatant.

4. **Tissue homogenate**:
1) Take an appropriate amount of tissue blocks, wash with pre-chilled PBS to remove blood, weigh, and set aside (if the tissue blocks are large, cut them into smaller pieces before homogenizing);
2) Multiple homogenization methods can be used simultaneously to achieve better disruption: first, transfer the tissue blocks into a glass homogenizer, add 5-10 mL of pre-chilled PBS for thorough grinding, and perform this process on ice; the resulting homogenate can be further processed using ultrasonic disruption or repeated freeze-thaw cycles;
3) Centrifuge the prepared homogenate at 5000×g for 5 minutes and collect the supernatant.

5. Cell Lysate:
1) Adherent cells need to be first digested with trypsin, then centrifuged to collect the cells (suspension cells can be directly collected by centrifugation).
2) Wash the collected cells three times with cold PBS.
3) Lyse the cells using physical methods (ultrasonic disruption of cells can be performed first, followed by repeated freeze-thaw cycles).
4) Centrifuge the sample at 1500 × g for 10 minutes at 4°C, and collect the supernatant for further use.

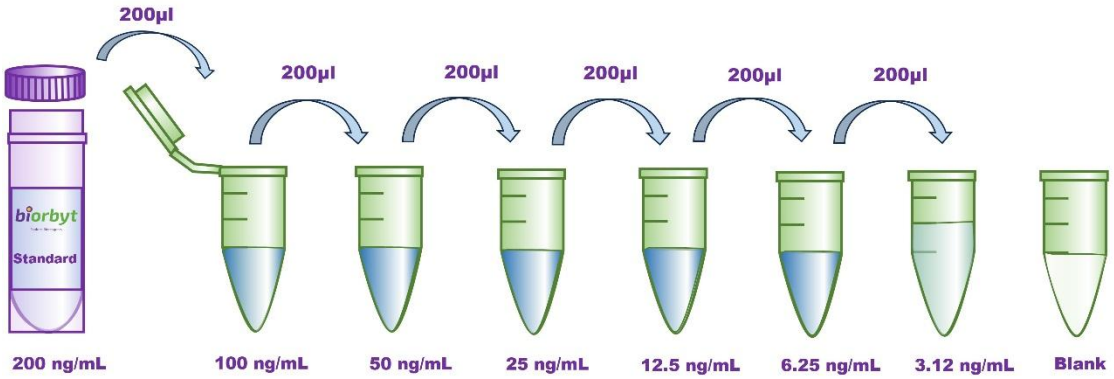
6. If the sample cannot be tested immediately, dispense it according to the minimum amount of use, and store it in -20℃ to -70℃ to avoid repeated freezing and thawing. Avoid haemolytic or hyperlipidaemia samples.

Reagent Preparation

1. Reagent reheating: Please reheat the reagent kit and the sample to be tested at room temperature within 30 minutes before the test.

2. Preparation of Washing Buffer: Dilute the concentrated Washing Buffer (30×) to Washing Buffer working solution (1×) with double distilled water or deionized water, and keep it as standby.

3. Gradient dilution of standard: Take 1 ml of Standard/Sample Dilution Buffer (S1) into the lyophilized standard, allow it to stand for 15 min until it is completely dissolved, then gently mix it with a concentration of 200 ng/ml, take 6 EP tubes, add 200ul Standard/Sample Dilution Buffer (S1) each EP tube, and dilute twice according to the following concentration: 100, 50, 25, 12.5, 6.25, 3.12 ng/ml were diluted. 200 ng/ml is the highest concentration of the standard curve, and the Standard/Sample Dilution Buffer (S1) is the zero point (0ng/ml) of the standard curve. The Standard Stock Solutions (1000ng/ml) that has not been used up should be discarded.



4. Detection Solution A and Detection Solution B: Before use, gently shake by hand a few times or briefly centrifuge to bring any liquid deposited on the tube walls or bottle cap down to the bottom of the tube. Just before use, dilute each solution 1:100 with the diluent (e.g., 10 µL of Detection Solution A in 990 µL of

diluent) and mix thoroughly. Prepare the total amount needed for each experiment (100 µL per well) based on prior calculations and prepare an extra 0.1–0.2 mL to ensure sufficient volume.

Operation Steps

1. Sample addition: According to the required amount for the experiment, take out the corresponding antibody-coated plates, and add **50 µl** per well of the prepared standards, standard zero point, and samples to be tested at the bottom of the wells.

2. Add Detection Solution A working solution (prepared freshly before use): add **50 µl** per well.

3. Incubation: Seal the plate with sealing tape and incubate at 37°C for 60 minutes.

4. Washing: Carefully remove the sealing tape, discard the liquid, flick dry, fill each well with **350 µl** washing solution, let stand for 1-2 minutes, then discard; repeat this process **3 times**, and finally blot dry on absorbent paper.

5. Add Detection Solution B working solution (prepared freshly before use): add **100 µl** per well.

6. Incubation: Seal the plate with sealing tape and incubate at 37°C for 60 minutes.

7. Washing: Follow the same washing procedure as described above (step 4), wash the plate **5 times**.

8. Color development: Add **90 µl** of substrate solution to each well, seal the plate, and incubate at 37°C for 15-25 minutes.

9. Termination: Add **50 µl** of stop solution to each well (the color changes from blue to yellow).

10. Measurement: Use a microplate reader to measure the absorbance (OD value) at 450 nm for each well. The measurement should be performed within 5 minutes after adding the stop solution.

Result Judgment

1. The OD value of each standard and sample minus the OD value of the blank well is the final value, and if a duplicate well is made, its mean value shall be calculated.

2. Take the absorbance OD value as the ordinate (Y) and the corresponding standard concentration as the abscissa (X) to generate the corresponding standard curve. The LPS content of the sample can be calculated from the standard curve according to its OD value. If the OD value of the sample is higher than the upper limit of the standard curve, perform the appropriate dilution during the test, and then multiply the concentration of the sample by the corresponding dilution.

Kit Performance

The difference between batches should be less than 10%

Detection Range

3.12 ng/ml -200 ng/ml

Sensitivity

1.24 ng/ml

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