LPS from *E. coli* (O111:B4) Detection Kit

Catalog # 6039

For Research Use Only - Not Human or Therapeutic Use

INTRODUCTION

Lipopolysaccharide (LPS) is a major component of the outer-membrane in gram-negative bacteria. While the main role of LPS is to maintain the integrity of the bacteria envelope, LPS also binds to the CD14/TLR4/MD2 receptor complex on many cell types (1,2), such as B cells, macrophages, and dendritic cells, resulting in pro-inflammatory cytokine release from these cells (3). This activation of the innate immune response causes inflammation which may play important roles in obesity and chronic inflammatory diseases such as Crohn's Disease, Chronic Fatigue Syndrome, Ulcerative Colitis, and Rheumatoid Arthritis. Translocation of bacteria toxins from intestinal flora may also trigger the pathogenesis of autoimmune diseases (4,5). To examine the possible link between pathogenic toxins from intestinal bacteria and autoimmune diseases, we provide an LPS (*E. coli* O111:B4) detection kit as well as ELISA kits for detecting staphylococcal enterotoxins A and B (Catalog # 6029 & 6030). For further requests and consultation, please contact info@amsbio.com.

Note: Because *E. coli* (O111:B4) may not be a natural resident in SPF mouse guts, inoculation of the *E. coli* or its LPS (i.e. immunization, oral feeding) may be required for animal studies. We recommend testing LPS levels in naive animals depending on animal vendors, animal strains, and housing conditions.

KIT COMPONENTS

Item	Quantity	Amount	Storage
LPS from *E. Coli* (O111:B4) Standard (60391)	1 vial	20 ng, lyophilized	-20°C
Detection Antibody (60393)	1 vial	50 µl	-20°C
Solution B - Sample/Standard Dilution Buffer (61027)	1 Bottle	50 ml	-20°C
Solution C - Detection Antibody Dilution Buffer (61025)	1 Bottle	20 ml	-20°C
Solution D – Streptavidin Peroxidase Dilution Buffer (9055)	1 Bottle	20 ml	-20°C
Streptavidin Peroxidase (9029)	2 vials	50 µl	-20°C
TMB (90023)	2 vials	0.2 ml	-20°C
Chromogen Dilution Buffer (90022)	1 Bottle	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid (9016)	1 Bottle	10 ml	-20°C
Wash Buffer, 20X (9005)	1 Bottle	50 ml	-20°C
Capture Antibody Coated 96-Well ELISA Plate (Purple)	1 each	8-well Strips x12	-20°C

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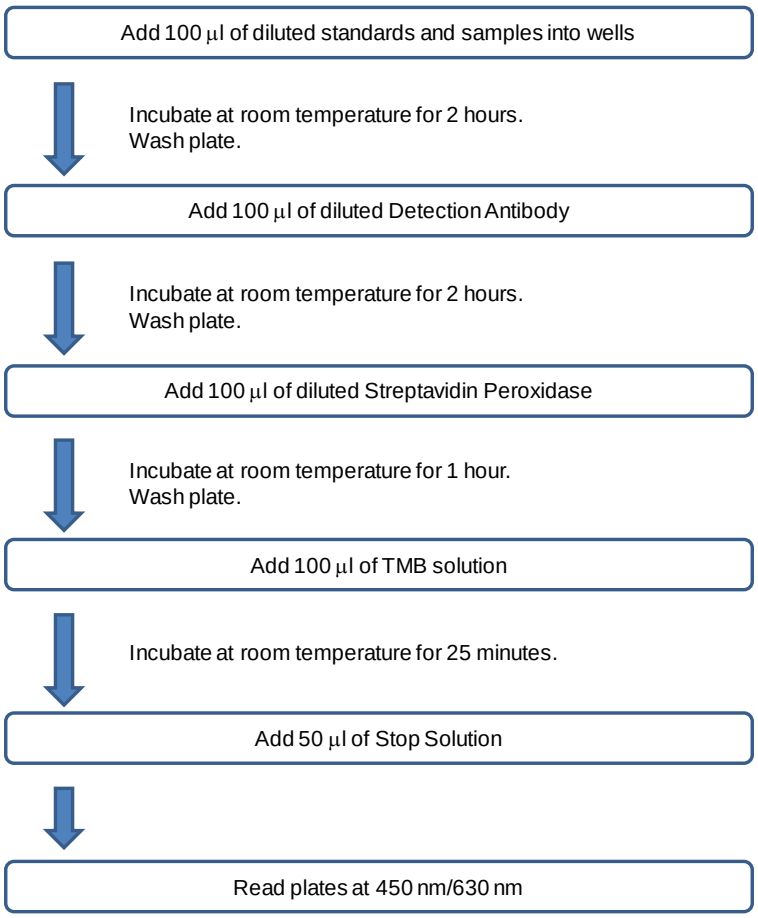
ASSAY OUTLINE



NOTES BEFORE USING ASSAY

Note 1: It is recommended that the standard and samples be run in duplicate.

Note 2: Warm up all buffers to room temperature before use.

Note 3: Partially used reagents may be kept at –20°C.

Note 4: Crystals may form in Wash Buffer, 20X when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are dissolved completely.

Note 5: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

Note 6: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

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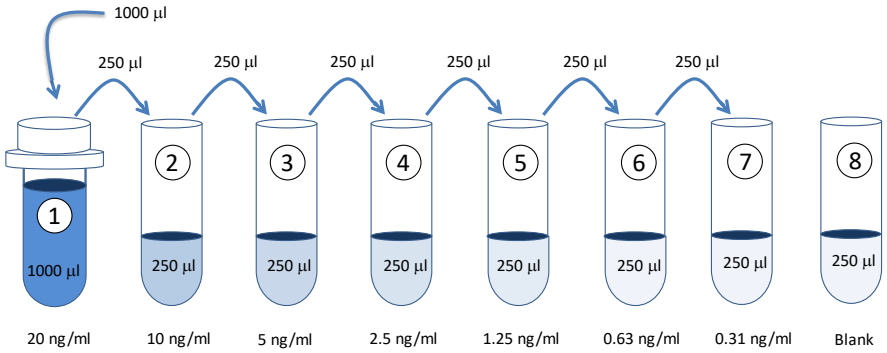
ASSAY PROCEDURE

1. **Prepare Standard Dilutions**: The recommended standard range is 0.31 - 20 ng/ml. Dissolve one vial of LPS standard in 1 ml of Sample/Standard Dilution Buffer (Solution B) for the 20 ng/ml standard. Then serially dilute it with Solution B. For example, mix 250 µl of the standard (20 ng/ml) with an equal volume of Solution B to make a 10 ng/ml solution, and then repeat it five more times for 5, 2.5, 1.25, 0.63, and 0.31 ng/ml solutions. The remaining 20 ng/ml standard stock may be stored at –20°C for use in a second assay. We recommend making fresh serial dilutions for each assay.



2. **Prepare Sample Dilutions:** Centrifuge samples at 10,000 rpm at 4°C for 3 minutes to remove insoluble materials and lipids. Dilute the supernatants with an equal volume of Solution B. For example, take 100 µl of supernatant, and mix with 100 µl of Solution B. If the LPS level is higher than 20 ng/ml, re-assay the sample at a higher dilution.

   Note 1: Buffer used to prepare the original samples may be contaminated with LPS. We recommend assaying the buffer to avoid overestimated assay results.

   Note 2: Dilute samples at least 1:1 with Solution B depending on the estimated LPS level in the samples. Two to three different sample dilutions are recommended if the LPS levels in the samples are unknown.

3. **Add Standards and Samples**: Add 100 µl of Solution B (blank), standards, and samples to designated wells in duplicate according to the layout in Figure 1. Incubate at room temperature for 2 hours.

Figure 1 - A Standard Assay Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	20	20	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	10	10	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	5	5	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	2.5	2.5	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	1.25	1.25	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	0.63	0.63	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	0.31	0.31	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	B	B	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

Standards Samples

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4. **Dilute Wash Buffer**: Dilute 50 ml of Wash Buffer, 20X in 950 ml of distilled water (1X wash buffer). Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

5. **Add Detection Antibody Solution**: Prepare detection antibody solution with Detection Antibody Dilution Buffer (Solution C) as shown in the following table.

Strip #	Detection Antibody (µl)	Solution C (ml)
2	8.5	1.7
4	17	3.3
6	25	5.0
8	33	6.6
10	41	8.2
12	50	10.0

Add 100 µl of detection antibody solution to each well and incubate at room temperature for 2 hours.

6. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

7. **Add Streptavidin Peroxidase Solution**: Prepare streptavidin peroxidase solution with Streptavidin Peroxidase Dilution Buffer (Solution D) as shown in the following table.

Strip #	Streptavidin Peroxidase (µl)	Solution D (ml)
2	17	1.7
4	33	3.3
6	50	5.0
8	66	6.6
10	82	8.2
12	100	10.0

Add 100 µl of streptavidin peroxidase solution to each well and incubate at room temperature for 1 hour.

8. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

9. **Add TMB Solution**: Use new tubes when preparing TMB solution. Just prior to use, prepare TMB solution with Chromogen Dilution Buffer as shown in the following table.

Strip #	TMB (µl)	Chromogen Dilution Buffer (ml)
2	34	1.7
4	66	3.3
6	100	5.0
8	132	6.6
10	164	8.2
12	200	10.0

Add 100 µl of TMB solution to each well immediately after washing the plate and incubate for 25 minutes at room temperature

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10. **Stop**: Stop the reaction with 50 µl of 2N Sulfuric Acid (Stop Solution) to each well.

11. **Read Plate**: Read the OD values at 450 nm. If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution. A 630 nm filter can be used as a reference.

CALCULATION OF RESULTS

1. Average the duplicate OD values for the blank, standards, and test samples.
2. Subtract the "blank" (B) values from the averaged OD values in step 1.
3. Plot the OD values of standards against the concentration of LPS (ng/ml). Using a log/log plot will linearize the data. Figure 2 shows a representative experiment where the standard range is 0.31-20 ng/ml.
4. The ng/ml of LPS in test samples can be calculated using regression analysis.

Figure 2 - A typical standard curve for LPS assay

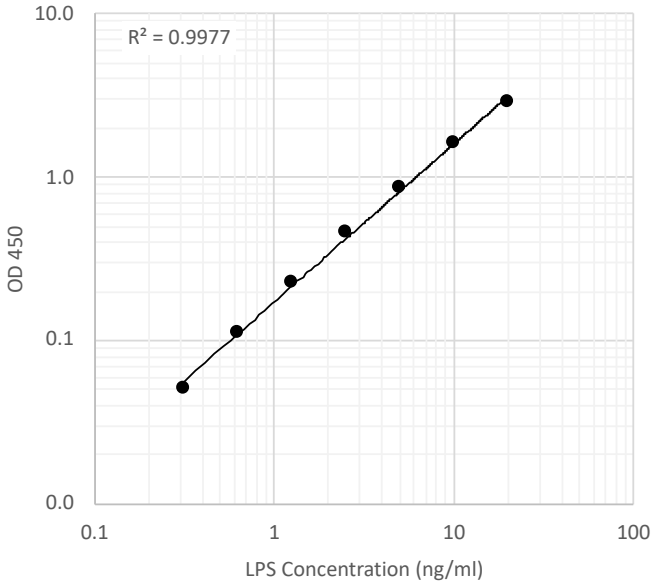

Table 1 - Reproducibility for LPS ELISA Kit

Test	0.63 ng/ml	2.5 ng/ml	10 ng/ml
Intra-Assay CV (%)	7.0	2.6	0.8
Inter-Assay CV (%)	7.8	4.6	0.1
Spike Test* (%)	109%	106%	101%

* Known amounts of LPS was added to samples and then diluted with Sample/Standard Dilution Buffer (Solution B).

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