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

Catalog # 6039

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kit to quantify *E. coli* (O111:B4) LPS levels in samples
FORMAT:	Pre-coated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	5.5 hours
STANDARD RANGE:	4 ng/ml to 0.06 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Serum and Plasma
RECOMMENDED SAMPLE DILUTIONS:	1:1 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C
VALIDATION DATA:	Intra-assay (2.6-6.6%)/Inter-assay (4.6-7.7%)/Spiking Test (106-110%)
NOTES:	

16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072

Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094

www.chondrex.com
support@chondrex.com

LPS from *E. coli* (O111:B4) Detection ELISA 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, Chondrex Inc. provides 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 visit www.chondrex.com or contact Chondrex, Inc. Customer Support at support@chondrex.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 or oral feeding) may be required for animal studies. Chondrex, Inc. recommend testing LPS levels in naive animals to determine baseline LPS levels that can result from different vendors, animal strains, and housing conditions.

KIT COMPONENTS

Item	Quantity	Amount	Storage
LPS from *E. Coli* (O111:B4) Standard (60391)	1 vial	4 ng, lyophilized	-20°C
Detection Antibody (60393)	1 vial	50 µl	-20°C
Solution B - Sample/Standard/Detection Antibody Dilution Buffer (67015)	1 Bottle	50 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

ASSAY OUTLINE

Add 100 µl of diluted standards and samples into wells

↓ Incubate at room temperature for 2 hours.
Wash plate.

Add 100 µl of diluted Detection Antibody

↓ Incubate at room temperature for 2 hours.
Wash plate.

Add 100 µl of diluted Streptavidin Peroxidase

↓ Incubate at room temperature for 1 hour.
Wash plate.

Add 100 µl of TMB solution

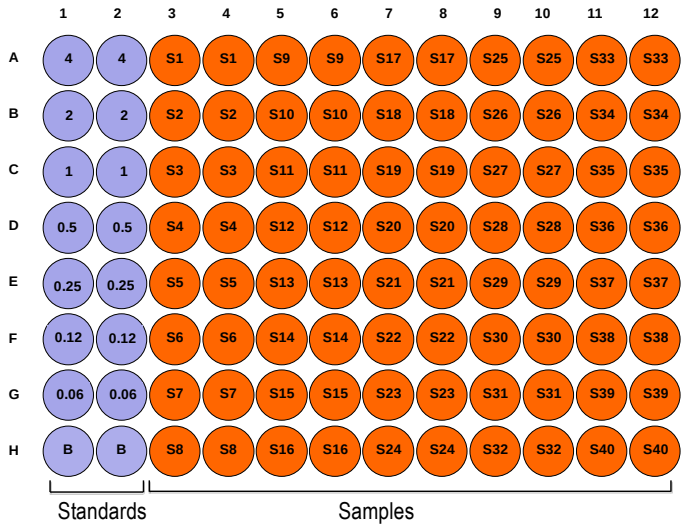
↓ Incubate at room temperature for 25 minutes.

Add 50 µl of Stop Solution

↓

Read plates at 450 nm/630 nm

PLATE LAYOUT



16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com

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: 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 completely dissolved.

NOTE 4: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

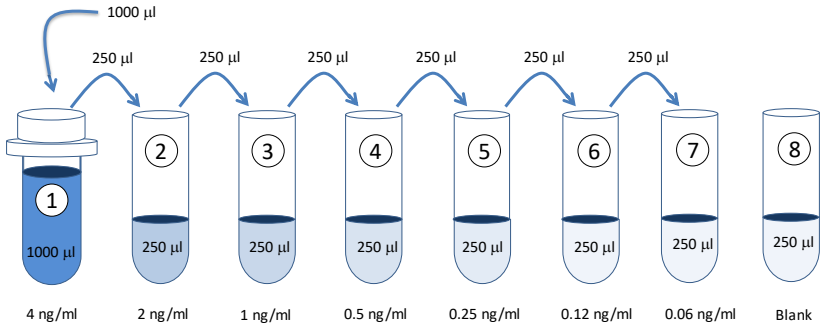
NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

NOTE 6: For partial reagent use, please see the assay protocol's corresponding step for the appropriate dilution ratio. For example, if the protocol dilutes 50 µl of a stock solution in 10 ml of buffer for 12 strips, then for 6 strips, dilute 25 µl of the stock solution in 5 ml of buffer. Partially used stock reagents may be kept in their original vials and stored at -20ºC for use in a future assay.

NOTE 7: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

ASSAY PROCEDURE

1. **Prepare Standard Dilutions**: The recommended standard range is 0.06 - 4 ng/ml. Dissolve one vial of LPS standard in 1 ml of Sample/Standard/Detection Antibody Dilution Buffer (Solution B) for the 4 ng/ml standard. Then serially dilute it with Solution B. For example, mix 250 µl of the standard (4 ng/ml) with an equal volume of Solution B to make a 2 ng/ml solution, and then repeat it five more times for 1, 0.5, 0.25, 0.12, and 0.06 ng/ml solutions. The remaining 4 ng/ml standard stock may be stored at –20°C for use in a second assay. Chondrex, Inc. recommends 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 4 ng/ml, re-assay the sample at a higher dilution.

   NOTE 1: Buffer used to prepare the original samples may be contaminated with LPS. Chondrex, Inc. recommends 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. Incubate at room temperature for 2 hours.

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**: Dilute one vial of Detection Antibody in 10 ml of Sample/Standard/Detection Antibody Dilution Buffer (Solution B). Add 100 µl of detection antibody solution to each well and incubate at room temperature for 2 hours.

Strip #	Detection Antibody (µl)	Solution B (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

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**: Dilute one vial of streptavidin peroxidase solution in 10 ml of Streptavidin Peroxidase Dilution Buffer (Solution D). Add 100 µl of streptavidin peroxidase solution to each well and incubate at room temperature for 1 hour.

Strip #	Streptavidin Peroxidase (µl)	Solution D (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

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. Dilute one vial of TMB in 10 ml of Chromogen Dilution Buffer. Add 100 µl of TMB solution to each well immediately after washing the plate and incubate for 25 minutes at room temperature.

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

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.

CALCULATING RESULTS

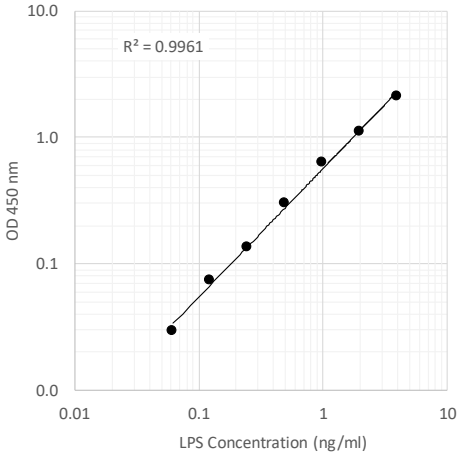
1. Average the duplicate OD values for the standards, blanks (B), and test samples.

2. Subtract the averaged blank OD values from the averaged OD values of the standards and test samples.

3. Plot the OD values of the standards against the ng/ml of antibody standard. Using a log/log plot will linearize the data. Figure 1 shows a representative experiment where the standard range is 0.06 - 4 ng/ml.

4. The ng/ml of antibody in test samples can be calculated using regression analysis. Multiply it by the sample dilution factor to obtain the antibody concentration (ng/ml) in original sample specimens.

Figure 1 - A Typical Standard Curve for the LPS Detection ELISA Kit



VALIDATION DATA

Table 1 - Reproducibility Data for the LPS Detection ELISA Kit

Test	0.13 ng/ml	0.5 ng/ml	2 ng/ml
Intra-Assay CV (%)	6.6	5.1	2.6
Inter-Assay CV (%)	5.2	7.7	4.6
Spike Test* (%)	110%	109%	106%

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

TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.'s ELISA FAQ for more information.

16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com

REFERENCES

1. Poltorak, X. He, I. Smirnova, M. Liu, H. Van, *et al*., Defective LPS Signaling in C3H/HeJ and C57BL/10ScCr Mice: Mutations in Tlr4 Gene. *Science* **282**, 2085-8 (1998).

2. R Shimazu, S. Akashi, H. Ogata, Y. Nagai, K. Fukudome, K. Miyake, *et. al*, MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. *J Exp Med* **189**,1777-82 (1999).

3. Ulmer, H. Flad, T. Rietschel, T. Mattern, Induction of Proliferation and Cytokine Production in Human T Lymphocytes by Lipopolysaccharide (LPS). *Toxicology* **152**, 37-45 (2000).

4. K. Terato, T. Waritani, R. Fukai, H. Shionoya, H. Itoh, K. Katayama, *et al*., Contribution of Bacterial Pathogens to Evoking Serological Disease Markers and Aggravating Disease Activity in Rheumatoid Arthritis. *PLoS One* **13**, e0190588 (2018).

5. K. Terato, C. Do, H. Shionoya, Slipping Through the Cracks: Linking Low Immune Function and Intestinal Bacterial Imbalance to the Etiology of Rheumatoid Arthritis. *Autoimmune Dis* **2015**, 636207 (2015).

16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com