

## Mouse anti-*Escherichia coli* Lipopolysaccharide (O111:B4) Antibody Assay Kits

Catalog # 6106, 6107, 6110, 6111

For Research Use Only - Not Human or Therapeutic Use

### INTRODUCTION

Recent studies indicate environmental factors, especially intestinal microbiota and their toxins, may play pathogenic roles in autoimmune diseases such as rheumatoid arthritis (RA) (1-7), inflammatory bowel diseases (IBDs) (8, 9), systemic lupus erythematosus (SLE) (10), and other chronic disorders (11-13). It is possible that increased translocation of bacteria and bacterial toxins associated with high mucosal permeability and low immune function may be the primary and common pathogenesis of these autoimmune disorders (14). Mice are ideal experimental animals to test this hypothesis due to the variety in genetic backgrounds, strains, and housing conditions, such as germ free, gnotobiotic, specific pathogen free, and conventional, all of which can affect susceptibility to potential pathogens. These differences in susceptibility may be attributed to the maturity of the immune system, depending on the environment.

To facilitate and promote studies that determine immune responses to environmental agents, Chondrex, Inc. provides mouse antibody ELISA kits against *Escherichia coli* O111:B4 lipopolysaccharide (*E. coli* LPS). In addition, Chondrex, Inc. also provides mouse antibody ELISA kits against *E. coli* O111:B4 (Catalog # 6206, 6207, 6209 - 6212), *Staphylococcal aureus* (Catalog # 6213), staphylococcal enterotoxin A (Catalog # 6218 - 6221), and staphylococcal enterotoxin B (Catalog # 6214 - 6217). For further requests or consultation, please contact us at support@chondrex.com.

#### Catalog #

Mouse anti-LPS IgG Antibody Assay Kit (catalog # 6106)  
 Mouse anti-LPS IgG1 Antibody Assay Kit (catalog # 6107)  
 Mouse anti-LPS IgG2a Antibody Assay Kit (catalog # 6110)  
 Mouse anti-LPS IgG2b Antibody Assay Kit (catalog # 6111)

### KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Antibody IgG (61066) IgG1 (61076) IgG2a (61106) IgG2b (61116)	1 vial	100 ng, lyophilized	-20°C
Secondary Antibody (peroxidase-conjugated polyclonal antibodies) IgG (61062) IgG1 (61072) IgG2a (61102) IgG2b (61112)	2 vials	50 µl/vial	-20°C
Solution A - Blocking Buffer (61026)	1 bottle	10 ml	-20°C
Solution B - Sample/Standard Dilution Buffer (61027)	1 bottle	50 ml	-20°C
Solution C - Secondary Antibody Dilution Buffer (61025)	1 bottle	20 ml	-20°C
TMB Solution (90023)	2 vials	0.2 ml/vial	-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
LPS (from <i>E. coli</i> O111:B4)-coated 8-Well Strips	12 each	8-well strips	-20°C

An antigen uncoated plate (Catalog #9026) is not included. Please contact us at support@chondrex.com to place an order.



- 4. Prepare Sample Dilutions:** Add 10  $\mu\text{l}$  of mouse serum sample to 990  $\mu\text{l}$  of Solution B (1:100), and keep it as a stock solution for future assays. Then, further dilute the sample with Solution B depending on the antibody levels. For example, take 200  $\mu\text{l}$  of the sample stock solution and mix with 200  $\mu\text{l}$  of solution B to make a 1:200 dilution. If it is necessary to assay antibodies at a low dilution of less than 1:200 due to low antibody levels, antigen uncoated control strips will be necessary. Please contact support@chondrex.com.

Note: Chondrex, Inc. recommends running a preliminary assay using various dilutions of sera (1:200, 1:1,000, 1:5,000) in order to determine the optimal dilution of your samples, especially before assaying a large number of samples.

- 5. 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 blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*
- 6. Add Standards and Samples:** Add 100  $\mu\text{l}$  of standards, Solution B (blank), and samples to wells in duplicate according to the desired layout (Figure 1a or Figure 1b). Incubate at room temperature for 2 hours.

Note: If a sample with a dilution of 1:200 or less is assayed, add 100  $\mu\text{l}$  of the diluted samples to the antigen-coated strips (S1) and the corresponding uncoated strips (SB1) as mapped out in Figure 1b.

- 7. 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 blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*
- 8. Add Secondary Antibody:** Dissolve one vial of Secondary Antibody in 10 ml of Secondary Antibody Dilution Buffer (Solution C). Add 100  $\mu\text{l}$  of secondary antibody solution to each well and incubate at room temperature for 1 hour.
- 9. 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 blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*
- 10. Add TMB:** Use new tubes when preparing TMB. Dilute one vial of TMB with 10 ml of Chromogen Dilution Buffer just prior to use. Add 100  $\mu\text{l}$  of the TMB solution to all wells immediately after washing the plate and incubate at room temperature for 25 minutes.
- 11. Stop:** Add 50  $\mu\text{l}$  of 2N sulfuric acid (Stop Solution) to each well.
- 12. Read Plate:** Read the OD values at 450 nm (a 630 nm filter can be used as a reference). If the OD values of the samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution.

## CALCULATION OF ANTIBODY TITERS

1. Average the duplicate OD values for the blanks (BL), test samples and standards.
2. Subtract the blank values from the averaged OD values of the test samples and standards respectively.

NOTE: If the antigen uncoated wells were used, subtract the OD values of samples tested in uncoated wells (background values) from their counterpart OD values in antigen coated wells from step 2 to eliminate values associated with non-specific reactions.

3. Plot the OD values of standards against the ng/ml of standard. A log/log plot will linearize the data. Figure 2 shows a representative experiment where the standard range is from 1.6 to 100 ng/ml for LPS.
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 2 - Typical standard curves for mouse anti-LPS IgG ELISA.

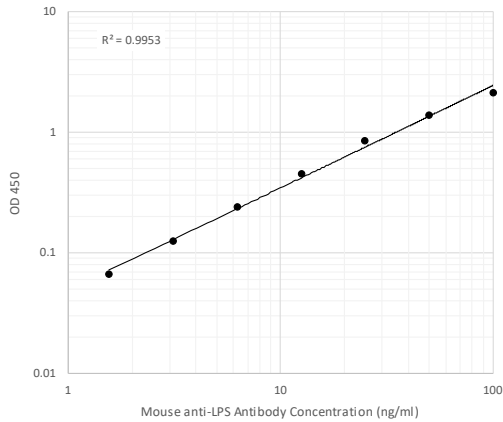


Table 1 - Reproducibility data for Mouse Anti-LPS IgG Antibody Assay Kit

Test At	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Inter-Assay CV (%)	11.7	4.0	2.0
Intra-Assay CV (%)	7.0	6.0	0.9
Spiking Test*	93%	105%	106%

Table 2 - Reproducibility data for Mouse Anti-LPS IgG1 Antibody Assay Kit

Test At	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Inter-Assay CV (%)	9.7	13.1	9.3
Intra-Assay CV (%)	8.8	4.5	3.6
Spiking Test*	108%	107%	106%

Table 3 - Reproducibility data for Mouse Anti-LPS IgG2a Antibody Assay Kit

Test At	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Inter-Assay CV (%)	6.1	4.8	2.6
Intra-Assay CV (%)	2.3	1.0	3.1
Spiking Test*	98%	99%	88%

Table 4 - Reproducibility data for Mouse Anti-LPS IgG2b Antibody Assay Kit

Test At	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Inter-Assay CV (%)	1.9	9.8	8.1
Intra-Assay CV (%)	5.4	3.1	6.9
Spiking Test*	106%	110%	115%

\* Standard was added with known amounts of antibody and then diluted with Sample/Standard Dilution Buffer to assay anti-LPS antibodies by ELISA.

## REFERENCES

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