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

Catalog # 6206, 6207, 6210, 6211

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). Increased translocation of bacteria and bacterial toxins associated with high mucosal permeability and low immune function may play roles in the primary and common pathogenesis of these autoimmune disorders (14). Mice are ideal experimental animals to study this hypothesis due to the variety in genetic backgrounds, strains, and housing conditions (germ free, gnotobiotic, specific pathogen free, or conventional), which can affect susceptibility to potential pathogens. The variability of susceptibility to pathogens may contribute 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 (*E. coli*). In addition, Chondrex, Inc. also provides mouse antibody ELISA kits against lipopolysaccharide from *E. coli* O111:B4 (Catalog # 6106, 6107, 6110, 6111), *Staphylococcus aureus* (Catalog # 6213), Staphylococcal enterotoxin A (Catalog # 6218 - 6221), and Staphylococcal enterotoxin B (Catalog # 6214 - 6217). For further requests or consultation, please contact support@chondrex.com.

Catalog #

Mouse anti-*E. coli* IgG Antibody Assay Kit (Catalog # 6206)
Mouse anti-*E. coli* IgG1 Antibody Assay Kit (Catalog # 6207)
Mouse anti-*E. coli* IgG2a Antibody Assay Kit (Catalog # 6210)
Mouse anti-*E. coli* IgG2b Antibody Assay Kit (Catalog # 6211)

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Antibody IgG (62066) IgG1 (62076) IgG2a (62106) IgG2b (62116)	1 vial	100 ng, lyophilized	-20°C
Secondary Antibody (peroxidase-conjugated polyclonal antibodies) IgG (62062) IgG1 (62072) IgG2a (62102) IgG2b (62112)	2 vials	50 µl/vial	-20°C
Solution A - Blocking Buffer (62026)	1 bottle	10 ml	-20°C
Solution B - Sample/Standard Dilution Buffer (62027)	1 bottle	50 ml	-20°C
Solution C - Secondary Antibody Dilution Buffer (62025)	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
E. coli O111:B4-coated 8-Well Strips	12 each	8-well strips	-20°C

An antigen un-coated plate (Catalog #9026) is not included. Please contact support@chondrex.com to place an order.

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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 the 20X wash buffer 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.

PLATE MAPPING

Map the plate based on the number of samples and sample dilution. For example, if sample dilution is higher than 1:200, use the standard layout (Figure 1a), but if sample dilution is lower than 1:200, antigen un-coated wells should be used to determine the background noise reaction OD values of individual samples (Figure 1b).

Figure 1a - Standard layout of antigen coated wells.

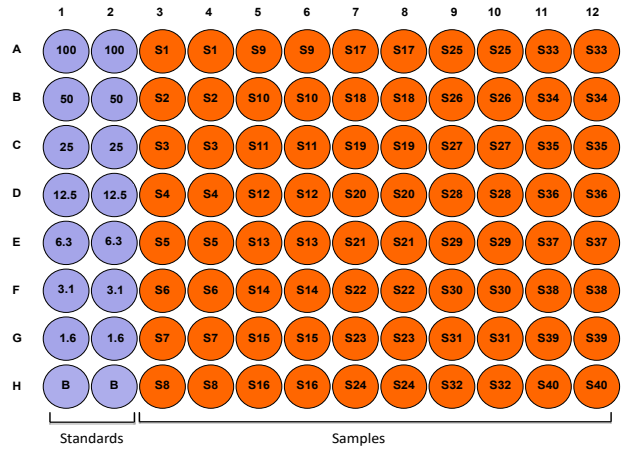
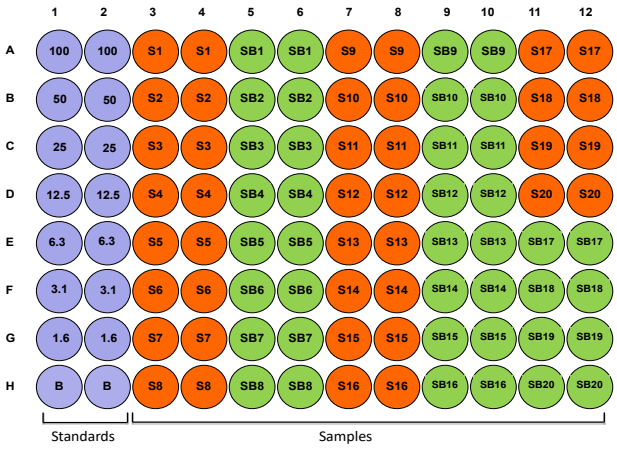


Figure 1b - Standard layout of antigen coated and un-coated wells.



ASSAY PROCEDURE

1. **Add Blocking Buffer:** Add 100 µl of the Blocking Buffer (Solution A) to each well and incubate for 1 hour at room temperature

Note: If a sample with a dilution of 1:200 or less is assayed, antigen un-coated strips should be used. Chondrex, Inc provides antigen un-coated plates (Catalog #9026). Contact support@chondrex.com for more information. Solution A must be added to the un-coated wells without prior washing because any contaminants in the vessel containing the washing buffer will bind to the strips. For example, add 100 µl of Solution A to the antigen-coated strips (S1) and the corresponding un-coated strips (SB1) as mapped out in Figure 1b.

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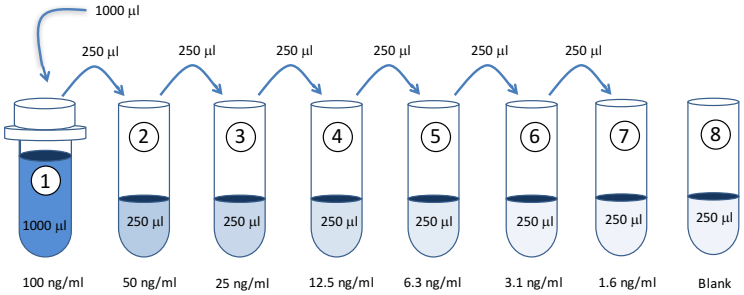
2. **Prepare Standard Dilutions:** Dissolve one vial of standard (100 ng/vial) with 1 ml Standard/Sample dilution buffer (Solution B) to make a 100 ng/ml stock standard solution. Prepare standard serial dilutions by mixing 250 µl of the stock standard solution with 250 µl of Solution B (50 ng/ml). Repeat this procedure to make 25, 12.5, 6.3, 3.1, and 1.6 ng/ml standard solutions for a total of 7 serial standard dilutions. Keep the remaining 100 ng/ml stock standard solution in its original vial at -20°C for future assays.



3. **Prepare Sample Dilutions:** Add 10 µl of mouse serum sample to 990 µl 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 µl of the sample stock solution and mix with 200 µl 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 for more information.

   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.

4. **Wash**: Dilute 50 ml of 20X wash buffer 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 blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

5. **Add Standards and Samples**: Add 100 µl 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 µl of the diluted samples to the antigen-coated strips (S1) and the corresponding uncoated strips (SB1) as mapped out in Figure 1b.

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 blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

7. **Add Secondary Antibody**: Dissolve one vial of Secondary Antibody in 10 ml of Secondary Antibody Dilution Buffer (Solution C). Add 100 µl of secondary antibody 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 blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

9. **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 µl of the TMB solution to all wells immediately after washing the plate and incubate at room temperature for 25 minutes.

10. **Stop**: Add 50 µl of 2N sulfuric acid (Stop Solution) to each well.

11. **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.

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CALCULATION OF ANTIBODY TITERS

1. Average the duplicate OD values for the blanks (B), 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 *E. coli* .

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 curve for mouse anti-*E. coli* IgG ELISA.

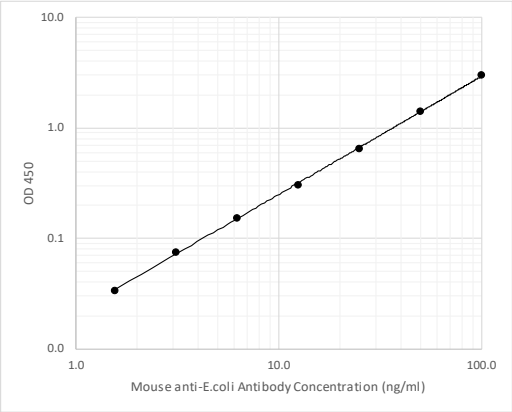

Table 1 - Reproducibility data for Mouse Anti-*E. coli* IgG Antibody Assay Kit

Test At	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Inter-Assay CV (%)	9.5	1.8	1.9
Intra-Assay CV (%)	4.4	3.0	8.5
Spiking Test*	99%	100%	99%

Table 2 - Reproducibility data for Mouse Anti-*E. coli* IgG1 Antibody Assay Kit

Test At	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Inter-Assay CV (%)	4.1	2.2	2.9
Intra-Assay CV (%)	2.5	1.1	1.4
Spiking Test*	103%	99%	101%

* Known amounts of antibody were added to standard amounts and then diluted with Sample/Standard Dilution Buffer to assay anti-*E. coli* antibodies by ELISA.

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Table 3 - Reproducibility data for Mouse Anti-*E. coli* IgG2a Antibody Assay Kit

Test At	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Inter-Assay CV (%)	7.1	5.3	2.1
Intra-Assay CV (%)	1.5	1.0	2.2
Spiking Test*	111%	100%	100%

Table 4 - Reproducibility data for Mouse Anti-*E. coli* IgG2b Antibody Assay Kit

Test At	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Inter-Assay CV (%)	7.2	7.4	7.1
Intra-Assay CV (%)	8.7	4.9	3.1
Spiking Test*	97%	98%	106%

* Known amounts of antibody were added to standard amounts and then diluted with Sample/Standard Dilution Buffer to assay anti-*E. coli* antibodies by ELISA.

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