Mouse anti-Staphylococcal Enterotoxin B (SEB) Antibody Assay Kits

Catalog #6214, 6215, 6216, 6217

For Research Use Only - Not Human or Therapeutic Use

INTRODUCTION

Staphylococcus aureus (S. *aureus*) is a gram-positive coccal bacterium frequently found in the human respiratory tract and on the skin. S. *aureus* infections contribute to a broad spectrum of illnesses, including endocarditis, septic arthritis (2), toxic-shock syndrome, scalded-skin syndrome (3), and food poisoning (1, 4). Recently, antibiotic-resistant strains (e.g. methicillin-resistant S. *aureus*) are being isolated more frequently which presents a challenge to clinical medicine.

This bacterium produces a variety of toxic exoproteins that can trigger massive pro-inflammatory cytokine release, leading to severe inflammation and tissue damage (5-7). Among these toxins, staphylococcal enterotoxins A and B (SEA and SEB) are known superantigens that bridge MHC-class II molecules and T-cell receptors, resulting in a potent stimulation of the host immune system, which is believed to contribute to the pathogenicity of autoimmune diseases (8). For instance, a study found that the presence of anti-SEA and anti-SEB IgE antibodies correlated with the severity of skin lesions in children with atopic dermatitis (9). SEA and SEB have also been used in vaccine development to protect against staphylococcal infections (5,6). Anti-SEA and/or SEB IgG antibodies may work as vaccine-induced, allergen-specific IgG antibodies to occupy the binding sites intended for IgE antibodies.

To study the pathological roles of SEA and SEB in mice, Chondrex, Inc. provides mouse anti-SEA IgG antibody and IgG subtype antibody ELISA kits (Catalog # 6218 - 6221) and mouse anti-SEB IgG antibody and IgG subtype antibody ELISA kits (Catalog # 6214 - 6217). In addition, Chondrex, Inc. also provides a *S. aureus* IgG antibody ELISA kit (Catalog # 6213), as well as antibody ELISA kits for the gram-negative bacterium *Escherichia coli* (O111:B4) (Catalog # 6206, 6207, 6209 - 6212) and its toxin, LPS (Catalog # 6106, 6107, 6110, 6111). For further requests and consultation, please contact Chondrex, Inc. at support@chondrex.com.

Antigen Color Coding - Catalog #

Antigen	Color coding	IgG	IgG1	IgG2a	IgG2b
S. aureus	Blue	6213	-	-	-
SEA	Yellow	6218	6219	6220	6221
SEB	Green	6214	6215	6216	6217

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Antibody IgG (62141) IgG1(62151) IgG2a (62161) IgG2b (62171)	1 vial	100 ng, lyophilized	-20°C
Secondary Antibody (Peroxidase-Conjugated Goat Anti-Mouse Polyclonal Antibodies) IgG (62143) IgG1(62153) IgG2a (62163) IgG2b (62173)	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 containing DMSO (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
SEB-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.

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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 the sample dilution is more than 1:200, it is not necessary to run antigen uncoated wells (Figure 1a), but if the sample dilution is less than 1:200, it may be necessary to run antigen uncoated wells (sample blank (SB), green colored wells) to determine the background noise reaction OD values of individual samples (Figure 1b).

Figure 1a - Standard layout of antigen coated plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	100	100	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	50	50	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	25	25	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	12.5	12.5	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	6.3	6.3	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	3.1	3.1	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	1.6	1.6	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

Figure 1b - Standard layout of antigen coated and uncoated plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	100	100	S1	S1	SB1	SB1	S9	S9	SB9	SB9	S17	S17
B	50	50	S2	S2	SB2	SB2	S10	S10	SB10	SB10	S18	S18
C	25	25	S3	S3	SB3	SB3	S11	S11	SB11	SB11	S19	S19
D	12.5	12.5	S4	S4	SB4	SB4	S12	S12	SB12	SB12	S20	S20
E	6.3	6.3	S5	S5	SB5	SB5	S13	S13	SB13	SB13	SB17	SB17
F	3.1	3.1	S6	S6	SB6	SB6	S14	S14	SB14	SB14	SB18	SB18
G	1.6	1.6	S7	S7	SB7	SB7	S15	S15	SB15	SB15	SB19	SB19
H	B	B	S8	S8	SB8	SB8	S16	S16	SB16	SB16	SB20	SB20

Standards Samples

ASSAY PROCEDURE

1. **Dilute Wash Buffer:** Dilute 50 ml of 20X wash buffer in 950 ml of distilled water (1X wash buffer).

2. **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 assying samples with a dilution of 1:200 or less, antigen uncoated strips should be used. Solution A must be added to the uncoated wells without prior washing because contaminants in the vessel containing the washing buffer will bind to the wells. For example, add 100 µl of Solution A to the antigen-coated strips (S1, orange) and the corresponding uncoated strips (SB1, green) as mapped out in Figure 1b. Incubate for 1 hour at room temperature. Next, follow the standard assay protocol.

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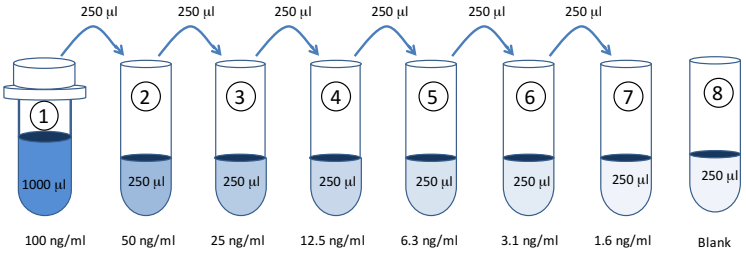
3. **Prepare Standard Dilutions:** The recommended standard range is 1.6-100 ng/ml. Dissolve one vial of standard (100 ng/vial) in 1 ml Standard/Sample Dilution Buffer (Solution B) to make a 100 ng/ml stock standard solution. Then, serially dilute it with Solution B. For example, mix 250 µl of the 100 ng/ml solution with an equal volume of Solution B to make a 50 ng/ml solution, and then repeat it five more times for 25, 12.5, 6.3, 3.1, and 1.6 ng/ml standard solutions. The leftover standard stock may be kept at -20°C for future assays



4. **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 diluted to less than 1:200, antigen uncoated control strips will be necessary. Please contact support@chondrex.com for more information.

   Note: It is recommended that a preliminary assay is run using various dilutions of sera (1:200, 1:1,000, 1:5,000) in order to determine the optimal dilution of the 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 µl of standards, Solution B (blank), and samples to wells in duplicate according to the choosen layout (see Figures 1a or 1b). Incubate at room temperature for 2 hours.

   Note: If assying samples diluted to 1:200 or less, 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.

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**: Dilute one vial of Secondary Antibody with 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.

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

11. **Stop**: Add 50 µl 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.

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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 antigen uncoated wells were used, subtract the OD values of samples in uncoated wells (sample blank 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 1.6 to 100 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 2 - Typical standard curves for mouse anti-SEB IgG ELISA.

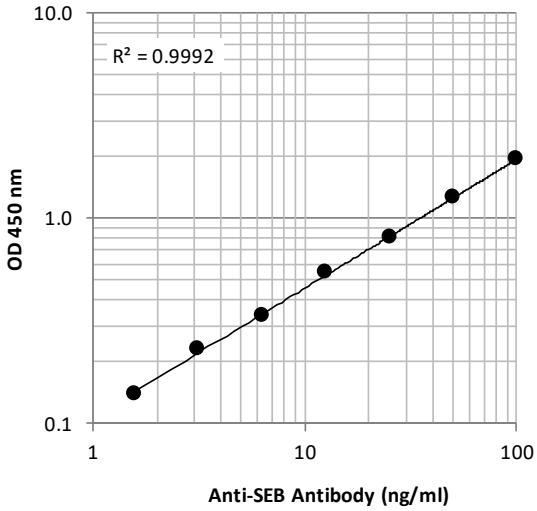

Table 1 - Reproducibility data for Mouse Anti-SEB IgG Antibody Assay Kit

Test At	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Inter-Assay CV (%)	4.6	1.5	4.5
Intra-Assay CV (%)	2.3	7.8	10.7
Spiking Test*	87.6%	82.2%	88.4%

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

Test At	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Inter-Assay CV (%)	1.5	7.7	8.8
Intra-Assay CV (%)	10.0	8.5	9.2
Spiking Test*	86.2%	87.6%	90.8%

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

Test At	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Inter-Assay CV (%)	9.8	3.0	7.0
Intra-Assay CV (%)	8.9	7.5	7.6
Spiking Test*	95.6%	90.8%	95.6%

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

Test At	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Inter-Assay CV (%)	4.9	8.1	5.4
Intra-Assay CV (%)	2.6	8.6	7.3
Spiking Test*	99.9%	88.7%	94.1%

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

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