Mouse Anti-Staphylococcal Enterotoxin A (SEA) Antibody ELISA Kits

Catalog # 6218-6221

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kits to quantify mouse anti-SEA IgG/IgG1/IgG2a/IgG2b antibodies
	6218: Mouse Anti-SEA IgG Antibody ELISA Kit
	6219: Mouse Anti-SEA IgG1 Antibody ELISA Kit
	6220: Mouse Anti-SEA IgG2a Antibody ELISA Kit
	6221: Mouse Anti-SEA IgG2b Antibody ELISA Kit
FORMAT:	Pre-coated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Indirect ELISA
ASSAY TIME:	4.5 hours
STANDARD RANGE:	100 ng/ml to 1.6 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) diluted samples/plate and up to 20 (duplicate) low dilution samples/plate
SAMPLE TYPES:	Serum and Plasma
RECOMMENDED SAMPLE DILUTIONS:	1:200 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C
VALIDATION DATA:	6218: Intra-assay (4.2-7.3%)/Inter-assay (1.4-10%)/Spiking Test (96-100%)
	6219: Intra-assay (3.3-10%)/Inter-assay (3.3-8%)/Spiking Test (98-107%)
	6220: Intra-assay (2.7-7.1%)/Inter-assay (1.4-2%)/Spiking Test (89-107%)
	6221: Intra-assay (5.7-8.2%)/Inter-assay (1.3-7.2%)/Spiking Test (95-103%)
NOTES:	N/A

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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, toxic-shock syndrome, scalded-skin syndrome, 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 (Cat # 6218 - 6221) and mouse anti-SEB IgG antibody and IgG subtype antibody ELISA kits (Cat # 6218 - 6221). In addition, Chondrex, Inc. also provides a variety of antibody ELISA kits against various bacteria and toxins. Please visit www.chondrex.com for more information. For further requests and consultation, please contact Chondrex, Inc. at support@chondrex.com.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Antibody: IgG (62181), IgG1(62191), IgG2a (62201), IgG2b (62211)	1 vial	100 ng, lyophilized	-20°C
Secondary Antibody (Peroxidase-Conjugated Goat Anti-Mouse Polyclonal): IgG (62183), IgG1(62193), IgG2a (62203), IgG2b (62213)	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
SEA-coated 8-Well Strips (Yellow)	12 each	96 well (8-well strips x12)	-20°C

An antigen uncoated plate (Cat # 9026) for lower sample dilutions is not included. Please contact support@chondrex.com to place an order.

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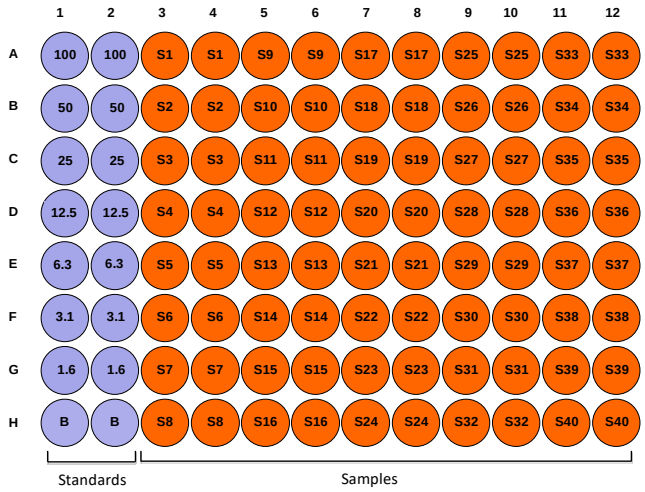
IDENTIFICATION OF ANTIGEN-COATED STRIPS

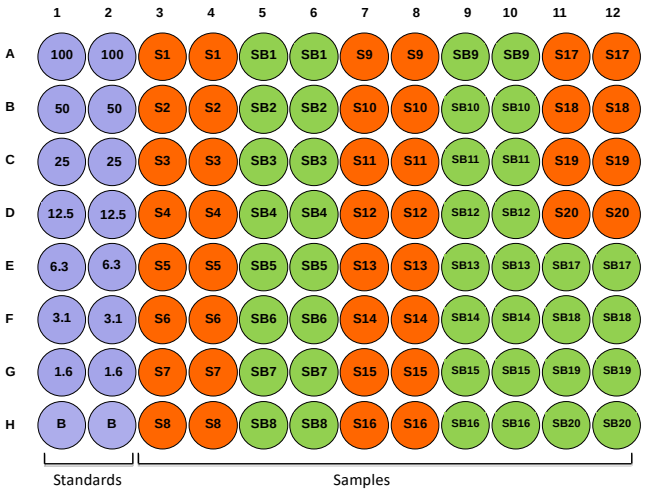
Antigens	Color Coding	IgG	IgG1	IgG2a	IgG2b
S. aureus	Blue	6213	-	-	-
SEA	Yellow	6214	6215	6216	6217
SEB	Green	6218	6219	6220	6221

PLATE MAPPING

Standard Layout of Antigen-Coated Plate



Standard Layout of Antigen-Coated and Uncoated Plate



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 -20°C 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.

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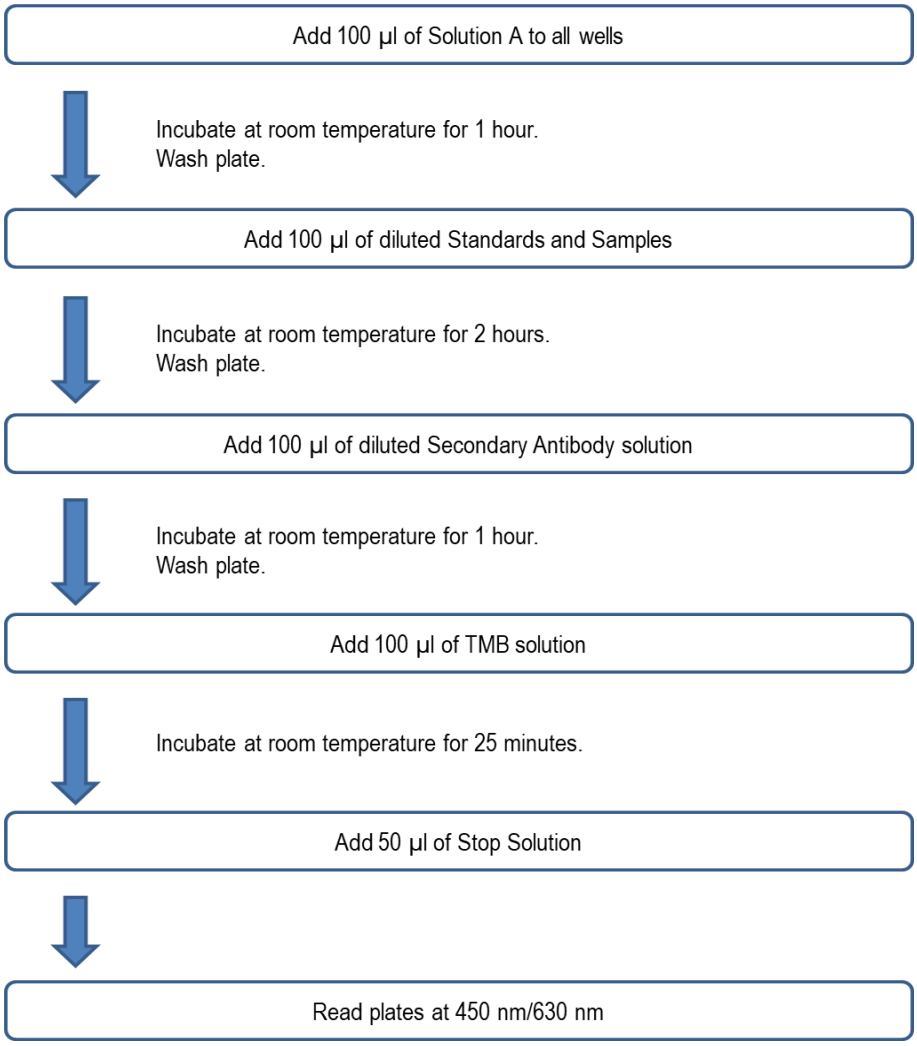
ASSAY OUTLINE



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 non-coated strips should be used. Solution A must be added to the non-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 uncoated strips (SB1). Incubate for 1 hour at room temperature.

2. **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 ml 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 use.

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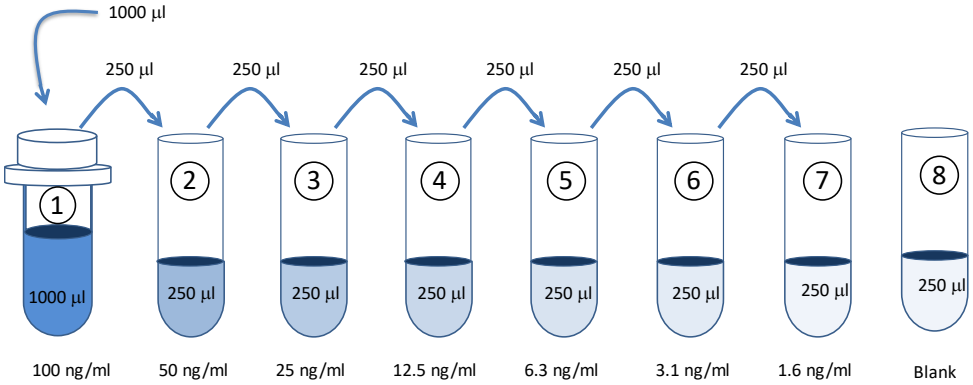

3. **Prepare Sample Dilutions:** Add 10 µl of mouse serum sample to 990 µl of Solution B (1:100) and keep it as a stock solution for future assays. Then, further dilute the sample with Solution B depending on the antibody levels. For example, take 200 µl of the sample stock solution and mix with 200 µl of Solution B to make a 1:200 dilution. If it is necessary to assay antibodies at 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. **Dilute Wash Buffer**: 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 Solution B (blank), standards, and samples to designated wells in duplicate. Incubate at room temperature for 2 hours.

   NOTE: If a sample with a dilution of 1:100 or less is assayed, add 100 µl of the diluted samples to the antigen-coated strips (S1) and the corresponding uncoated strips (SB1).

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**: Prepare the secondary antibody solution with Secondary Antibody Dilution Buffer (Solution C) as shown in the following table. Add 100 µl of secondary antibody solution to each well and incubate at room temperature for 1 hour.

Strip #	Secondary Antibody (µl)	Solution C (ml)
2	9	1.8
4	17	3.4
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. Just prior to use, prepare TMB solution with Chromogen Dilution Buffer as shown in the following table. The prepared TMB cannot be stored for the next assay. 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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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. A 630 nm filter can be used as a reference. If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution.

CALCULATING RESULTS

1. Average the duplicate OD values for the blank, standards, and test samples.

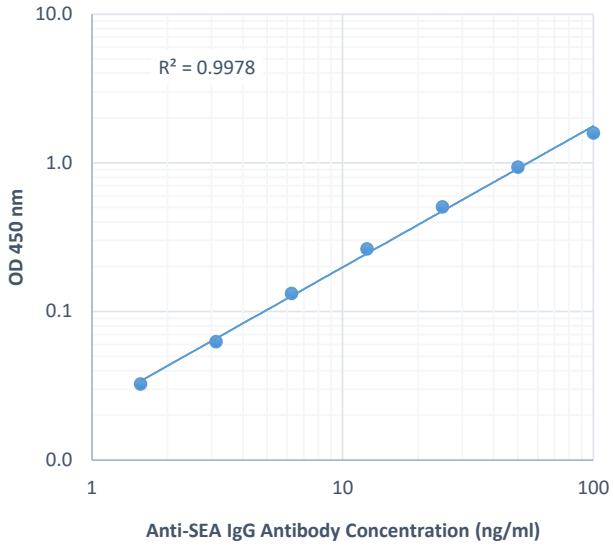
2. Subtract the "blank" (B) values from the averaged OD values in step 1.

   NOTE: If 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 concentration of standard antibody (ng/ml). Using a log/log plot will linearize the data. Figure 1 shows a representative experiment where the standard range is 1.6 - 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 1 - A Typical Standard Curve for the Mouse Anti-SEA IgG Antibody ELISA Kit



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VALIDATION DATA

Table 1 - Reproducibility data for the Mouse Anti-SEA IgG Antibody ELISA Kit

Test	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Intra-Assay CV (%)	4.2	7.3	6.5
Inter-Assay CV (%)	1.4	7.1	10.0
Spiking Test*	96%	100%	96%

Table 2 - Reproducibility data for Mouse Anti-SEA IgG1 Antibody ELISA Kit

Test	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Intra-Assay CV (%)	3.3	4.3	10.0
Inter-Assay CV (%)	8.0	6.2	3.3
Spiking Test*	106%	100%	98%

Table 3 - Reproducibility data for Mouse Anti-SEA IgG2a Antibody ELISA Kit

Test	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Intra-Assay CV (%)	7.1	4.7	2.7
Inter-Assay CV (%)	1.7	2.0	1.4
Spiking Test*	89%	95%	107%

Table 4 - Reproducibility data for Mouse Anti-SEA IgG2b Antibody ELISA Kit

Test	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Intra-Assay CV (%)	7.8	8.2	5.7
Inter-Assay CV (%)	1.3	7.2	3.0
Spiking Test*	103%	95%	98%

* Known amounts of anti-SEA antibodies were added to standards and then diluted with Sample/Standard Dilution Buffer to assay anti-SEA antibodies

TROUBLESHOOTING

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

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