Staphylococcal Enterotoxin A and B Detection Kits

Catalog # 6029 and 6030

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kits to quantify SEA and SEB in samples
FORMAT:	Pre-coated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
TIME:	1.5 hours
STANDARD RANGE:	10 ng/ml to 0.16 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Liquid samples
RECOMMENDED SAMPLE DILUTIONS:	1:1 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C
VALIDATION DATA:	6029: Intra-Assay (1.3-3.7%)/Inter-Assay (4.1-7.8%)/Spiking Test (92-103%)
	6030: Intra-Assay (1.2-2%)/Inter-Assay (1.1-8.6%)/Spiking Test (98-100%)
NOTES:	

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INTRODUCTION

The contamination of pathogenic microorganisms and their toxins in food and water is a serious issue for human health and safety (1, 2). For instance, enterotoxins (SEs) produced by *Staphylococcus aureus* (*S. aureus*) are heat-stable, meaning pathological activity remains even after exposure to sterilization techniques and digestive proteases. Among the SEs, staphylococcal enterotoxin A (SEA) and B (SEB) are confirmed toxins which cause enteritis and food poisoning. Symptoms include nausea, vomiting, diarrhea, which in severe cases, may lead to fatalities in children and the elderly (3-5). These SEs, known as superantigens, non-specifically activate T-cells, leading to proliferation which ultimately results in T-cell elimination. This activation directly and indirectly induces a massive release of inflammatory cytokines (6).

In addition to acute poisoning, researchers reported that these toxins may play roles in the pathogenesis of autoimmune diseases. More specifically, intestinal dysbiosis (enteromicrobial imbalance) was found in patients with rheumatoid arthritis (RA) and which may overwhelm the host immune defense functions by chronic exposure to excess amounts of these pathogens (7, 8). In animal models, SEs synergistically play a role in the pathogenesis of autoimmune-related diseases (9), such as atopic dermatitis (8, 10), food allergies (11, 12), colitis (13, 14), arthritis (11, 15-17), and systemic lupus erythematosus (11, 18).

Several methods exist for detecting pathogenic microorganisms and their toxins, such as polymerase chain reaction PCR), mass spectrometry, biosensor-based techniques, reversed passive latex agglutination, and enzyme-linked immunosorbent assay (ELISA) (5, 19). Of these techniques, PCR is ideal to detect the presence of microorganisms through their DNA or RNA. On the other hand, ELISA is widely used for detecting toxins in samples because of its high assay sensitivity and simplicity. For example, ELISAs were used to assay SEB in food samples as well as synovial fluids, sera, and urine from RA patients (6, 19).

Chondrex, Inc. provides ELISA kits for detecting SEA and SEB (Catalog# 6029, 6030) in food, feces, intestinal fluids, and other liquid samples. Chondrex, Inc. also provides mouse anti-SEA and SEB IgG antibody and IgG subtype antibody ELISA kits and a *S. aureus* IgG antibody ELISA kit. For further requests and consultation, please contact us at support@chondrex.com or visit www.chondrex.com.

KIT COMPONENTS

Item	Quantity	Amount	Storage
SEA Standard (60291) SEB Standard (60301)	1 vial	10 ng, lyophilized	-20°C
SEA Detection Antibody (60293) SEB Detection Antibody (60303)	1 vial	50 µl	-20°C
Solution B - Sample/Standard/Detection Antibody Dilution Buffer (67015)	1 bottle	50 ml	-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 (SEA: Yellow, SEB: Green)	1 each	8-well strips x 12	-20°C

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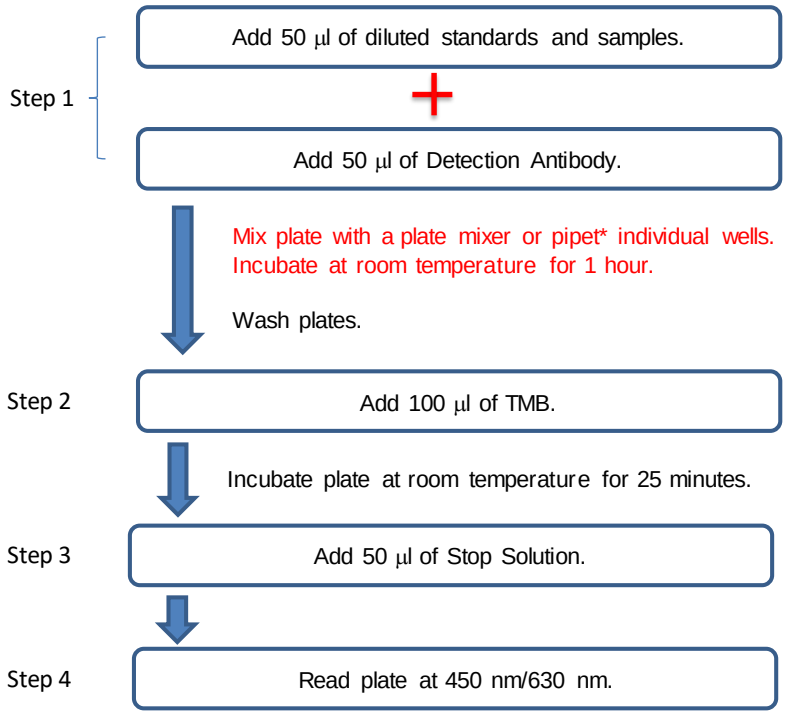
ASSAY OUTLINE



* Use one pipet tip per sample or standard. Do not cross-contaminate samples or standards by re-using pipet tips. A multi-channel pipet is recommended.

PLATE LAYOUT

	1	2	3	4	5	6	7	8	9	10	11	12
A	10	10	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	5	5	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	2.5	2.5	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	1.25	1.25	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	0.63	0.63	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	0.31	0.31	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	0.16	0.16	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	B	B	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40
	Standards		Standards									

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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: 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.

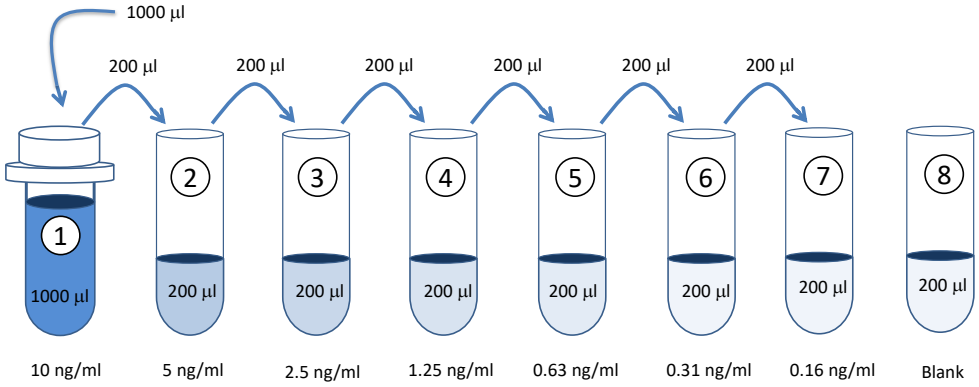
NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

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 0.16-10 ng/ml. Dissolve one vial of standard in 1 ml of Sample/Standard/Detection Antibody Dilution Buffer (Solution B) for the 10 ng/ml standard. Then serially dilute it with Solution B. For example, mix 200 ml of the standard (10 ng/ml) with an equal volume of Solution B to make a 5 ng/ml solution, and then repeat it five more times for 2.5, 1.25, 0.63, 0.31, and 0.16 ng/ml solutions. The remaining 10 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 SEA or SEB level is higher than 10 ng/ml, re-assay the sample at a higher dilution.

3. **Prepare Detection Antibody**: Dilute one vial in 5 ml of Sample/Standard/Detection Antibody Dilution Buffer (Solution B).

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Strip #	Detection Antibody (µl)	Solution B (ml)
2	8	0.8
4	17	1.7
6	25	2.5
8	33	3.3
10	42	4.2
12	50	5.0

4. **Add Standards and Samples**: Add Standards, Samples, and Detection Antibody: vortex individual standard, sample, and detection antibody tubes well. Add 50 µl of Solution B (blank), standards, and samples to designated wells. Add 50 µl of diluted detection antibody solution to all wells. Mix all wells by pipetting or with a plate shaker. Cover the plate with a plate sealer and incubate at room temperature for 1 hour.

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

6. **Add TMB Solution**: Use new tubes when preparing TMB solution. Just prior to use, dilute one vial of TMB solution with 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

7. **Stop**: Stop the reaction with 50 ml of 2N Sulfuric Acid (Stop Solution) to each well.

8. **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 representative experiments where the standard range is 0.16 -10 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.

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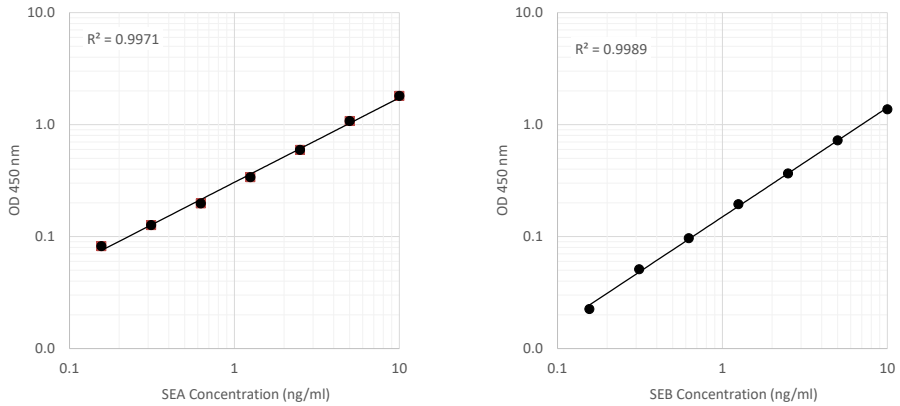
Figure 1- Typical Standard Curves for the SEA and SEB Detection ELISA Kits



VALIDATION DATA

Table 1 - Reproducibility Data for the SEA Detection ELISA Kit

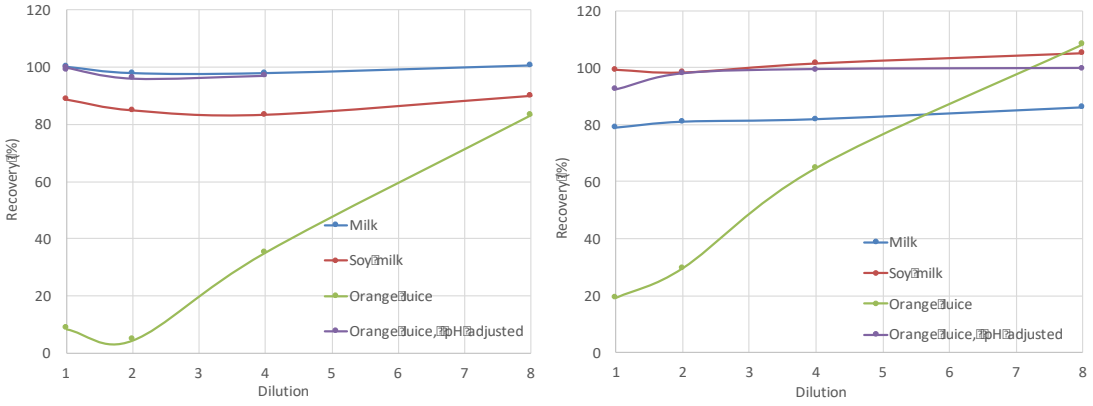
Test	0.32 ng/ml	1.25 ng/ml	5 ng/ml
Intra-Assay CV (%)	3.7	1.3	2.1
Inter-Assay CV (%)	7.8	7.2	4.1
Spike Test* (%)	100%	103%	92%

Table 2 - Reproducibility Data for the SEB Detection ELISA Kit

Test	0.32 ng/ml	1.25 ng/ml	5 ng/ml
Intra-Assay CV (%)	1.3	2.0	1.2
Inter-Assay CV (%)	1.1	3.6	8.6
Spike Test* (%)	99%	98%	100%

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

Figure 2 - Recovery Tests Using Liquid Samples (Left: SEA, Right: SEB). Samples needed to be adjusted to pH 7.5 before running the assay; best results are obtained if diluted with Solution B.



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TROUBLESHOOTING

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

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