Staphylococcal Enterotoxin A and B Detection Kit

Catalog # 6029, 6030

For Research Use Only - Not Human or Therapeutic Use

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 may cause overwhelming host 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 DNA or RNA detection. 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 for assaying 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 IgG antibody and IgG subtype antibody ELISA kits (Catalog # 6218 - 6221), mouse anti-SEB IgG antibody and IgG subtype antibody ELISA kits (Catalog # 6214 - 6217), and a *S. aureus* IgG antibody ELISA kit (Catalog # 6213). For further requests and consultation, please contact us at support@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	1 each	8-well strips x 12	-20°C

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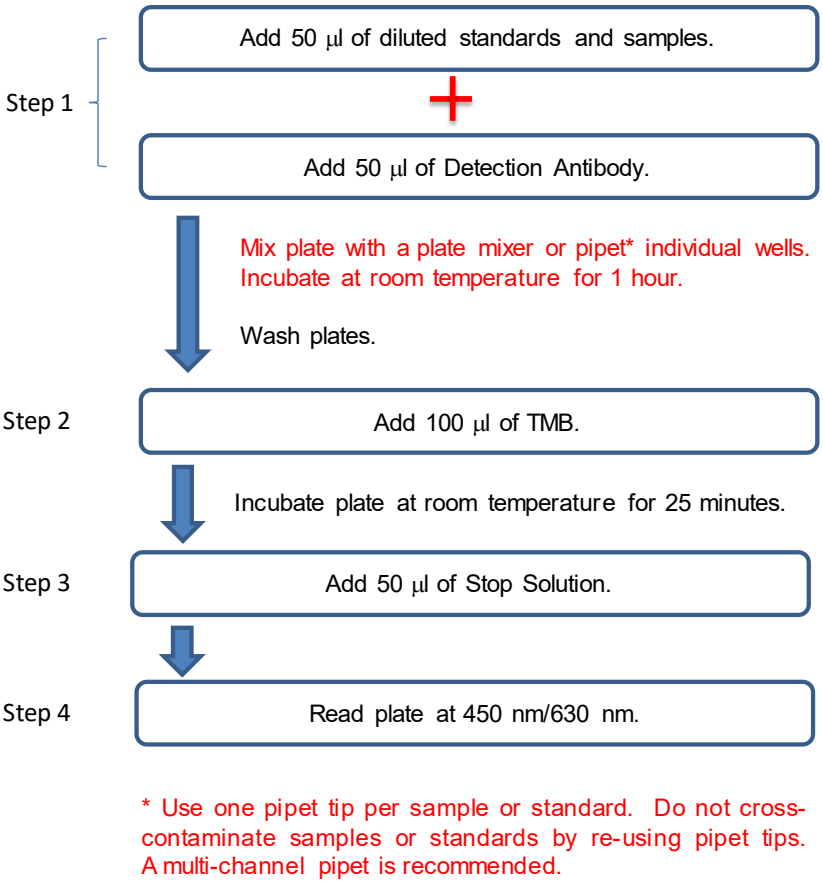
ASSAY OUTLINE



* 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.

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 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 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.

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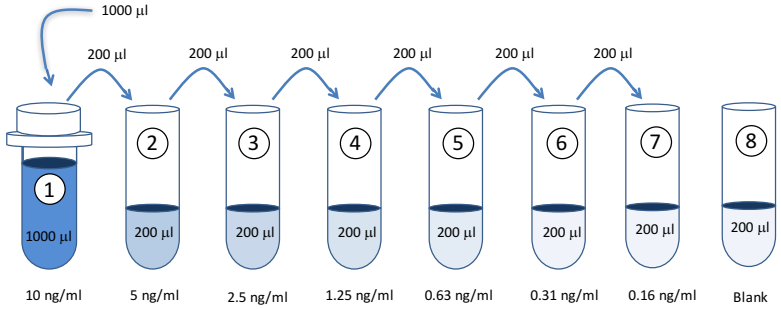
ASSAY PROCEDURE

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 µl 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. We recommend making fresh serial dilutions for each assay.



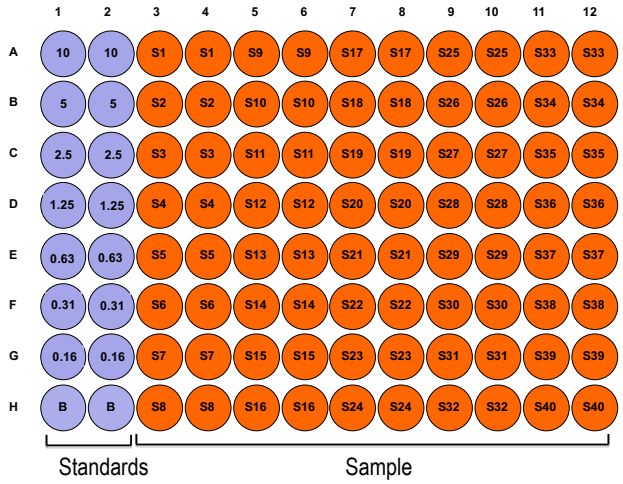
2. **Prepare Sample Dilutions**: Centrifuge samples at 10,000 rpm at 4°C for 3 minutes to remove insoluble materials and lipids. Dilute the supernatants with an equal volume of Solution B. For example, take 100 µl of supernatant, and mix with 100 µl of Solution B. If the SEA or SEB level is higher than 10 ng/ml, re-assay the sample at a higher dilution.

3. **Prepare Detection Antibody**: Prepare the detection antibody solution with Sample/Standard/Detection Antibody Dilution Buffer (Solution B) as shown in the following table.

Strip #	Detection Antibody (µl)	Solution B (ml)
2	8	0.9
4	17	1.7
6	25	2.5
8	33	3.3
10	41	4.1
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 appropriate wells (Figure 1). 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.

Figure 1 - A Standard Assay Layout



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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 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.

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

   Add 100 µl of TMB solution to each well immediately after washing the plate and incubate for 25 minutes at room temperature.

7. **Stop**: Stop the reaction with 50 µl 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.

CALCULATION OF 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.
3. Plot the OD values of standards against the concentration of SEA or SEB (ng/ml). Using a log/log plot will linearize the data. Figure 2 and Figure 3 show a representative experiment where the standard range is 0.16-10 ng/ml.
4. The ng/ml of SEA in test samples can be calculated using regression analysis.

Figure 2 - A typical standard curve for SEA assay

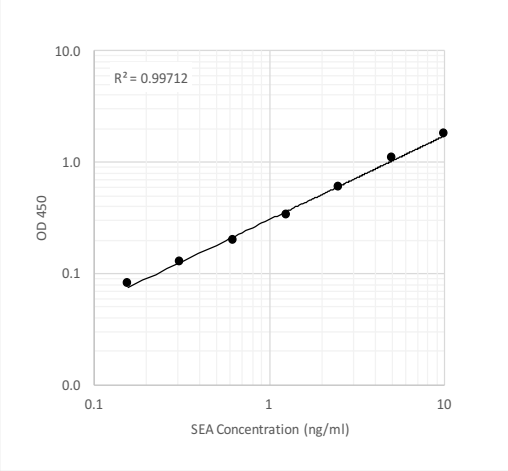

Figure 3 - A typical standard curve for SEB assay

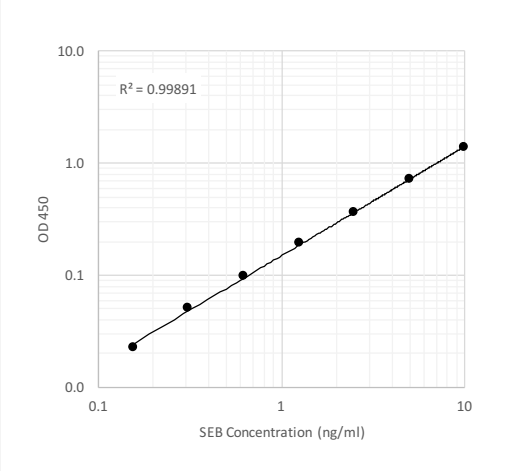

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Table 1 - Reproducibility for SEA ELISA Kit

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

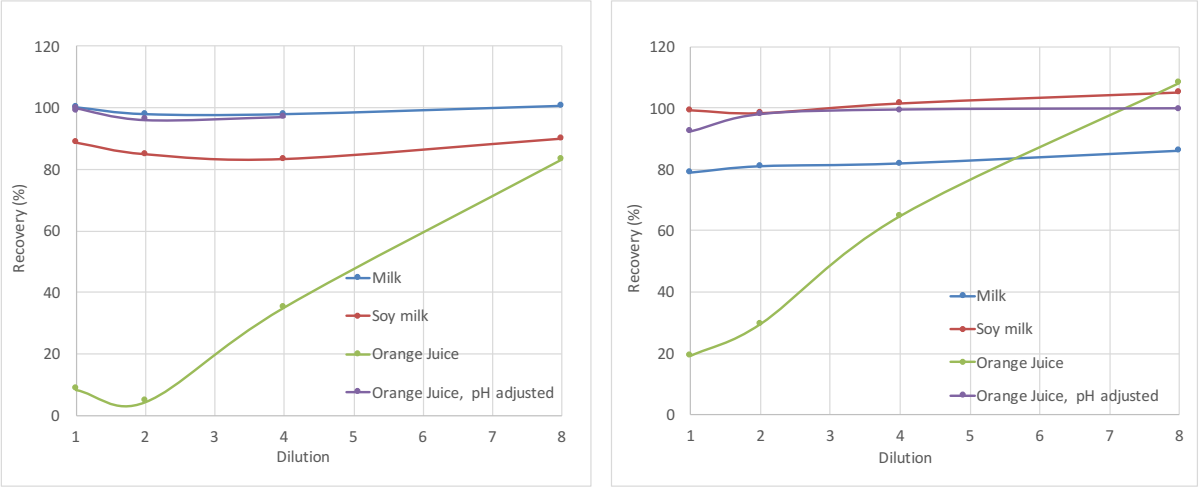
* Known amounts of SEA were added to samples and then diluted with Sample/Standard/Detection Antibody Dilution Buffer (Solution B).

Table 2 - Reproducibility for SEB ELISA Kit

Test	0.32 ng/ml	1.25 ng/ml	5 ng/ml
Inter-Assay CV (%)	1.1	3.6	8.6
Intra-Assay CV (%)	1.3	2.0	1.2
Spike Test*	98.8%	97.8%	99.3%

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

Figure 4. Recovery tests using liquid samples (Left: SEA , Right: SEB)



Samples have to be adjusted to pH 7.5 before assay.; best results are obtained if diluted with Solution B.

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