SET (Staphylococcal aureus Enterotoxin Total) ELISA Kit

Catalog No: E-FS-E118

96T/96T*3/96T*5

Version Number:	V1.3
Replace version:	V1.2
Revision Date:	2026.08.24

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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Test principle

This kit uses Sandwich-ELISA as the method for the qualitative detection. It can detect staphylococcal aureus enterotoxin (SET) (A-E and G-I) in samples, such as, milk, milk powder, and yogurt. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody working solution, control and other supplementary reagents. The microtiter plate in this kit has been pre-coated with coupled antibodies. The sample is added to the wells of the ELISA microtiter plate, and the SET in the sample is combined with the pre-coated antibody to form an SET-antibody compound. Free components are washed away. The Antibody Working Solution and HRP Conjugate are added to each well and react with the compound to form "antibody-SET-HRP conjugate" compound. The substrate reagent is added to initiate the color developing reaction. The presence of SET can be determined according to the OD value by using a microplate reader with 450 nm (630 nm) wavelength.

Technical indicator

Reaction mode (Incubation time and temperature)**:** 37℃; 60 min, 30 min, 15 min, 15 min.

Detection limit:

Sample type	Detection limit (ppb)				
	SEA	SEB	SEC	SED	SEE
Raw milk, sterilized milk, pasteurized milk, buffalo milk	0.75	0.25	0.15	2	0.75
Yogurt	3	1	0.6	8	3
Milk powder, whey powder, whey protein powder	4.5	1.5	0.9	12	4.5
Infant formula powder	6.75	2.25	1.35	18	6.75
Dairy beverage	1.5	0.5	0.3	4	1.5
Milk skin yogurt cheese, milk skin yogurt, cheese stick	3	1	0.6	8	3

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Antibody Working Solution	12 mL
HRP Conjugate	12 mL
Positive Control	2 mL
Negative Control	2 mL
Substrate Reagent A	7 mL
Substrate Reagent B	7 mL
Stop Solution	7 mL
Yogurt Extract	40 mL
20×Concentrated Wash Buffer	25 mL
Plate Sealer	1 piece
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Other materials required but not supplied

Instrument: Microplate reader, Homogenizer, Vortex mixer, Centrifuge, Balance (sensibility 0.01 g).

Micropipette: Single channel (20-200 µL, 100-1000 µL), Multichannel (30-300 µL).

Notes

1. The overall OD value will be lower when reagents have not been brought to room temperature before use or room temperature is below 25±2℃.
2. If the wells turn dry during the washing procedure, it will lead to bad linear standard curve and poor repeatability. Operate the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the reproducibility of this ELISA kit.
4. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid th e kit to strong light.
5. **Each reagent is optimized for use in the E-FS-E118. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E118 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue color. When OD value of standard (concentration: 0) < 0.8 unit (A450nm < 0.8), it indicates the reagent may be deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.
8. As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.
9. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
10. **For mentioned sample fast and efficient extraction methods are included in the kit descriptio n. Please consult technical support for the applicability if other sample need to be tested.**
11. The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS, etc. can be used for quantitative confirmation.

Storage and expiry date

Store the kit at 2-8℃. Do not freeze any test kit components.

Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2-8℃. After opening, the kit is stable for up to 1 month.

Expiry date: expiration date is on the packing box.

Experimental preparation

Restore all reagents and samples to room temperature before use.

Open the microplate reader in advance, preheat the instrument, and set the testing parameters

1. **Sample pretreatment Notice:**

   Experimental apparatus should be clean, and the pipette should be disposable to avoid cross-contamination during the experiment.

2. **Solution preparation**

   Please prepare solution according to the number of samples. Don't use up all components in the kit at once!

   Solution 1: **Wash Buffer**

   Dilute **20×Concentrated Wash Buffer** with deionized water. (20×Concentrated Wash Buffer (V): Deionized water (V) = 1 : 19).

3. **Sample pretreatment procedure**

3.1 Pretreatment of raw milk, sterilized milk, pasteurized milk, buffalo milk sample:

(1) Take 4 mL of sample into 10 mL centrifuge tube, centrifuge at 5000 r/min for 10 min at 4-10℃ (If a refrigerated centrifuge is not available, chill sample to approx 4-10℃ prior to centrifugation).

(2) Do not aspirate the upper fat layer, take 100 μL of middle layer for analysis.

Note: Sample dilution factor: 1

3.2 Pretreatment of yogurt, milk skin yogurt cheese, milk skin yogurt, cheese stick sample:

(1) Homogenize the sample completely, weigh 1±0.05 g of sample into 10 mL centrifuge tube, add 0.4 mL of **Yogurt Extract** and 2.6 mL of deionized water. Oscillate fully for 1 min.

(2) Centrifuge at 5000 r/min for 10 min at 4-10℃ (If a refrigerated centrifuge is not available, chill sample to approx 4-10℃ prior to centrifugation).

(3) Do not aspirate the upper fat layer, take 100 μL of middle layer for analysis.

Note: Sample dilution factor: 4

3.3 Pretreatment of milk powder, whey powder, whey protein powder sample:

(1) Weigh 1±0.05 g of sample into 10 mL centrifuge tube, add 5 mL of deionized water. Vortex thoroughly until completely dissolved.

(2) Centrifuge at 5000 r/min for 10 min at 4-10℃ (If a refrigerated centrifuge is not available, chill sample to approx 4-10℃ prior to centrifugation).

(3) Do not aspirate the upper fat layer, take 100 μL of middle layer for analysis.

Note: Sample dilution factor: 6

3.4 Pretreatment of infant formula powder sample:

(1) Weigh 1±0.05 g of sample into 10 mL centrifuge tube, add 8 mL of deionized water. Vortex thoroughly until completely dissolved.

(2) Centrifuge at 5000 r/min for 10 min at 4-10℃ (If a refrigerated centrifuge is not available, chill sample to approx 4-10℃ prior to centrifugation).

(3) Do not aspirate the upper fat layer, take 100 μL of middle layer for analysis.

Note: Sample dilution factor: 9

3.5 Pretreatment of dairy beverage sample:

(1) Weigh 1±0.05 g of sample into 10 mL centrifuge tube, add 0.4 mL of **Yogurt Extract** and 0.6 mL of deionized water. Oscillate fully for 1 min.

(2) Centrifuge at 5000 r/min for 10 min at 4-10℃ (If a refrigerated centrifuge is not available, chill sample to approx 4-10℃ prior to centrifugation).

(3) Do not draw up any impurities, take 100 μL of the supernatant for analysis.

Note: Sample dilution factor: 2

Assay procedure

Restore all reagents and samples to room temperature before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8℃.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. Set 2 wells for positive/negative control respectively. **Standard and Samples need test in duplicate.**
2. **Add Sample:**
   a) Add 100 μL of **Positive/Negative control** to positive/negative control well.
   b) Add 100 μL of **Sample** to sample well .
3. **Incubate:** Gently tap the plate to mix thoroughly, cover the plate sealer, incubate for 60 min at 37℃ in shading light.
4. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 260 μL of **Wash Buffer** (Solution 1) to each well and wash. Repeat the wash procedure for 5 times, 15-30 s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
5. **Antibody Working Solution:** Add 100 μL of **Antibody Working Solution** into each well. Gently oscillate for 10 s to mix thoroughly and cover the plate with plate sealer. incubate for 30 min at 37℃ in shading light.
6. **Wash:** repeat step 4.
7. **HRP Conjugate:** add 100 μL of **HRP Conjugate** to each well. Gently oscillate for 10 s to mix thoroughly, incubate for 15 min at 37℃ in shading light.
8. **Wash:** repeat step 4.
9. **Color Development:** add 100 μL of **Substrate mixed solution** to each well (**Substrate Reagent A** and **Substrate Reagent B** are fully mixed at ratio 1:1 by volume, the mixture should be used within 5 min, avoid using metal containers or stirring the reagents). Gently oscillate for 10 s to mix thoroughly. Incubate at 37℃ in for 15 min shading light (The reaction time can be extended according to the actual color change).
10. **Stop Reaction:** add 50 μL of **Stop Solution** to each well. Gently oscillate for 10 s to mix thoroughly.
11. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader. This step should be finished in 5 min after stop reaction.

Reference value

When, Average absorbance of negative control < 0.2 and the Average absorbance of positive control >0.5, results are valid. Otherwise, the test shall be repeated.

Interpretation of the results

Cut. Off= 0.2 + Average absorbance of negative control

1. **Positive result**: Average absorbance of sample ≥ Cut. Off.
2. **Negative result**: Average absorbance of sample < Cut. Off.

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