1st Edition, revised in November, 2020

SET (Staphylococcal aureus Enterotoxin Total) ELISA Kit

Catalog No: E-FS-E118

96T/96T*3

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.

Test principle

This kit uses Sandwich-ELISA as the method for the quantitative detection. It can detect staphylococcal aureus enterotoxin (SET) (A, B, C, D and E) 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 indicators

Reaction mode: 25℃; 60 min, 30 min, 15 min, 15 min.

Kit 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	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Acidity regulators	10 mL
10×Concentrated Wash Buffer	2*40 mL
Plate Sealer	3 pieces
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, Printer, Homogenizer, Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01 g).

High-precision transferpettor: Single channel (20-200 μL, 100-1000 μL).

Experimental preparation

Restore all reagents and samples to room temperature (25℃) 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

Solution 1: Wash Buffer

Dilute the **10×Concentrated Wash Buffer** with deionized water. (10×Concentrated Wash Buffer (V): Deionized water (V) =1:9).

3. Sample pretreatment

3.1 Pretreatment of milk sample:

(1) Take 10 mL of milk samples into 50 mL centrifuge tube, centrifuge at 10000 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) Take 100 μL of middle layer for analysis.

3.2 Pretreatment of yogurt sample:

(1) Weigh 1±0.05 g of yogurt samples into 50 mL centrifuge tube, add 5 mL of deionized water. Oscillate fully for 5 min.

(2) Centrifuge at 10000 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) Take all middle layer to another centrifuge tube, add **Acidity regulators** and adjust the pH to 7.0.

(4) Take 100 μL for analysis.

3.3 Pretreatment of milk powder sample:

(1) Weigh 1±0.05 g of milk powder samples into 50 mL centrifuge tube, add 7 mL of deionized water. Oscillate fully for 2 min.

(2) Take 100 μL for analysis.

Assay procedure

Restore all reagents and samples to room temperature (25℃) 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. **Positive/Negative Control and Samples need test in duplicate**
2. **Add sample:** add 100 μL of **Positive/Negative Control or Sample** per well, cover the plate with plate sealer. Oscillate for 5 s gently to mix thoroughly. Incubate at 37℃ for 60 min in shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 250 μL of **Wash Buffer** (Solution 1) to each well and wash. Repeat wash procedure for 5 times, 10 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).
4. **Antibody Working Solution**: add 100 μL **Antibody Working Solution**, cover the plate with plate sealer. Oscillate for 5 s gently to mix thoroughly. Incubate at 37℃ for 30 min in shading light.
5. **Wash:** repeat Step 3.
6. **HRP Conjugate:** add 100 μL of **HRP conjugate** to each well. Incubate at 37℃ for 15 min in shading light.
7. **Wash:** repeat Step 3.
8. **Color Development:** add 50 μL of **Substrate Reagent A** to each well, and then add 50 μL of **Substrate Reagent B**. Gently oscillate for 5 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).
9. **Stop reaction:** add 50 μL of **Stop Solution** to each well, oscillate gently to mix thoroughly.
10. **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

Normally, Average absorbance of negative control < 0.2 and the Average absorbance of positive control > 0.5.

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.

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. If the wells turn dry during the washing procedure, it will lead to 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. ELISA Microtiter plate should be covered by plate sealer. Avoid the 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. Stop solution is caustic, avoid contact with skin and eyes.
7. 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.
8. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
9. The kit is used for rapid screening of actual samples. If the test result is positive, the Micro-Organism method can be used 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℃.

Expiry date: expiration date is on the packing box.