TECHNICAL MANUAL

SET (Staphylococcal aureus Enterotoxin Total) ELISA Kit

- **SKU CODE:** FSES0094
- **SIZE:** 48T/96T
- **DETECTION PRINCIPLE:** Competitive
- **RUO:** Research-Use-Only

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SET (Staphylococcal aureus Enterotoxin Total) ELISA Kit

Please read entire manual carefully before starting experiment. DO NOT mix reagents and use reagents from different kits or batches to prevent assay failure.

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1. Key Features

Detection Method:

Sandwich

Detection limit:

-

Cross-reactivity:

-

Sample recovery rate:

-

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit and/or components as described in section 4. Date of expiration is on the ELISA Box label.

3. Product Description

The Assay Genie *Staphylococcus aureus* Enterotoxin Total (SET) Enzyme-Linked Immunosorbent Assay (ELISA) is a robust, qualitative screening tool based on the highly sensitive Sandwich-ELISA technique, specifically developed for the comprehensive detection of the five major enterotoxin serotypes (SET-A, B, C, D, and E) in complex food matrices such as milk, milk powder, and yogurt. The kit is supplied with essential components, including a microtiter plate pre-coated with high-affinity capture antibodies, specific Detection Antibody Working Solution, Horseradish Peroxidase (HRP) Conjugate, control standards, and supplementary reagents required for the procedure.

During the assay, target SET present in the prepared sample binds to the immobilized capture antibody on the plate, forming an initial immune complex, and all unbound components are removed via a wash step. Subsequently, the Detection Antibody Working Solution and HRP Conjugate are sequentially added to form a definitive "Capture Antibody–SET–HRP Conjugate" sandwich complex in each well. The addition of the Substrate Reagent then initiates a colorimetric reaction, catalyzed by the HRP, which is terminated by the Stop Solution. The presence of SET is qualitatively determined based on the resulting optical density (OD) value measured against the provided controls using a microplate reader at a primary wavelength of 450 nm, with an optional 630 nm reference wavelength for correction.

4. Kit Contents

No	Component Name	Specifications	Storage
1	ELISA Microtiter plate	96 wells	Store the kit at 2-8°C. Do not freeze any test kit components.
2	Antibody Working Solution	12 mL	
3	HRP Conjugate	12 mL	
4	Positive Control	2 mL	
5	Negative Control	2 mL	
6	Substrate Reagent A	6 mL	
7	Substrate Reagent B	6 mL	
8	Stop Solution	6 mL	
9	Acidity Regulators	10 mL	
10	10x Concentrated Wash Buffer	20 mL	
11	Plate Sealer	3 pieces	
12	Sealed Bag	1 piece	
13	Technical Manual	1 copy	

Additional materials required:

1. 37°C incubator.

2. Plate Reader with 450nm filter.

3. Precision pipettes and disposable pipette tips.

4. Distilled water.

5. Disposable tubes for sample dilution.

6. Absorbent paper.

5. Precautions

1. This kit is ideal for research purposes only and not for diagnostics or therapeutic uses.

2. To identify the concentration of your target, a pilot experiment using standards and a small number of samples is recommended.

3. Store all components as listed in this manual. Do not use the ELISA Kit after its expiration date

4. Ensure unopened and unused plate is kept dry to avoid contamination.

5. Before using the kit, centrifuge tubes to spin down standard & antibodies.

6. Wash steps are critical for the success of the assay, deviations from wash steps may cause false positives and result in a high background.

7. Duplicate wells are recommended for both standard and sample testing.

8. Do not let the microplate wells dry during assay.

9. Do not reuse tips and tubes to avoid cross contamination.

10. Avoid using the reagents from different batches together.

6. Experimental Preparation

The procedures outlined in this document are provided as general recommendations for sample preparation in ELISA assays. Due to the variability of biological samples and specific assay requirements, users are advised to optimize protocols based on their own experimental conditions.

***Note**: For information regarding validation data in specific samples, please contact our Technical Support Team at techsupport@assaygenie.com.*

Bring all reagents and samples to room temperature before use. Open the microplate reader in advance, preheat the instrument, and set the testing parameters.

A. Sample pretreatment Precautions

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

B. Solution preparation:

***Note:** 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 the 20xConcentrated Wash Buffer with deionized water.

(10xConcentrated Wash Buffer (V): Deionized water (V) =1:9).

C. Sample pretreatment procedure

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.

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. 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.

4. Pretreatment of whey powder sample:

(1) Weigh 1±0.05 g of whey powder samples into 50 mL centrifuge tube, add 4 mL of deionized water. Oscillate fully for 5 min.

(2) Centrifuge at 10000 r/min for 10 min.

(3) Take 100 µL of middle layer for analysis.

7. Assay Procedure

1. **Plate Setup:** Designate standard wells, test sample wells, and control (blank) wells on the pre-coated plate, and record their positions. It is recommended to measure each standard and sample in duplicate. ***Note:*** *Determine the optimal dilution ratio for samples through preliminary experiments or by following technical support recommendations*.
2. **Controls and Sample Addition:** Add 100 µL of Positive/Negative Control or Sample per well, cover the plate with plate sealer. Shake for 5 s gently to mix thoroughly. Incubate at 37℃ for 60 min protected from light.
3. **Washing:** Uncover the sealer carefully, remove the liquid of each well. Immediately add 250 µL of Wash Buffer (Solution 3). Repeat wash procedure for 5 times, 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).
4. **Antibody Working Solution:** Add 100 µL Antibody Working Solution, cover the plate with plate sealer. Shake for 5 s gently to mix thoroughly. Incubate at 37℃ for 30 min protected from light.
5. **Wash:** Repeat washing as indicated in step 3.
6. **HRP Conjugate:** add 100 µL of HRP Conjugate to each well, cover the plate sealer, oscillate for 10 s gently to mix thoroughly, incubate at 25°C for 30 min protected from light.
7. **Washing:** Repeat wash as instructed in step 3.
8. **Substrate Reagent:** Add 50 µL of Substrate Reagent A to each well, and then add 50 µL of Substrate Reagent B (1:1 ratio according to volume). Incubate 25°C for 15-20 min at in shading light. ***Note:*** *the reaction time can be shortened or extended according to the actual color change, but not more than 30 min. Preheat the Microplate Reader for about 15 min before OD measurement*.
9. **Stop Solution:** Add 50 µL of Stop Solution to each well and mix thoroughly. The colour will change to yellow immediately.

10. **OD Measurement:** Read the optical density (OD) at 450 nm in a microplate reader immediately after adding the Stop Solution.

8. Data Analysis

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

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.

9. ELISA Troubleshooting

Problem	Possible Causes	Solutions
Standard curve without signal	Incorrect reagent order; Mixed components from different kits; Missing reagents.	Ensure correct reagent order and use components from the same kit. Verify all reagents are added.
Overflow OD	Mixed components from different kits; Over-concentrated working solution	Use correct components and prepare solutions at recommended concentrations.
Poor standard curve	Incorrect curve fitting model.	Try alternative curve fitting models.
Samples without signal	Sample concentration too low; Incompatible buffer; Incorrect preparation; Sample degradation or excessive freeze-thaw.	Reduce dilution or concentrate sample. Check buffer compatibility and follow proper preparation and storage.
High CV%	Precipitate formation; Unclean plate; Foaming; Uneven washing; Incomplete reagent mixing; Pipetting inconsistency.	Dilute samples if needed, avoid foaming, ensure uniform washing, mix reagents thoroughly, and use calibrated pipettes.
Low standard signal	Improperly reconstituted standards; Degraded standards; Incorrect pipetting; Expired kit; Improper storage; Over-dried wells.	Reconstitute standards properly, use fresh kits, follow storage recommendations, and prevent wells from drying.
Slow colour development	TMB not equilibrated; Incorrect microplate reader wavelength; Over-washing.	Pre-warm TMB (30 min at 37°C), confirm correct wavelength (450 nm), and follow recommended washing times.
High background	Insufficient washing; Contaminated wash buffer; Excess detection reagents; Delayed reading; TMB exposed to light.	Wash adequately, prepare fresh wash buffer, use correct reagent amounts, read results promptly, and incubate TMB in the dark.

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.