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Cat No.: EKF1088

Staphylococcal aureus Enterotoxin Total (SET) ELISA Kit

If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

Email (Order) order@enkilife.com

Email (Techsupport) techsupport@enkilife.com

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Shelf life: Please refer to the label on the outer package.
Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Product description

This ELISA kit is rapid, qualitative enzyme-linked immunosorbent assays (ELISA) for the determination of Staphylococcus aureus Enterotoxin content in Food samples.

Key Features

- Sensitivity：0.15 ppb (ng/mL)
- Specificity：

SEA	20%
SEB	60%
SEC	100%
SED	7.5%
SEE	20%

- Detection limits：

Raw milk, sterilized milk, pasteurized milk, modified milk, yoghurt	0.15 ppb
Milk drink	0.3 ppb
Milk powder, whey powder, whey protein powder	0.9 ppb

Storage and Shelf Life

The kit should be stored at 2~8°C and must not be frozen. The shelf life of this product is 1 year. Do not use the kit beyond the expiration date.

Test Principle

This kit uses Sandwich-ELISA as the method for the qualitative detection. It can detect staphylococcal aureus enterotoxin (SET) in samples. 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.

Materials Supplied

Item	Quantity
Assay Plate	96T
Positive Control	1 x 2 mL
Negative Control	1 x 2 mL
Yoghurt extract	1 x 40 mL
HRP Conjugate	1 x 12 mL
Antibody	1 x 12 mL
TMB Substrate Reagent (A\B)	2 x 7 mL
Stop Solution	1 x 7 mL
Wash Buffer (20×)	1 x 25 mL
Plate Sealer	1 piece
Instruction Manual	1 piece
Sealed Bag	1 copy

Materials Required, Not Supplied

- Instruments: Microplate reader, printer, homogenizer, nitrogen blow-drying device, vortex, centrifuge, constant temperature incubator, balance (sensitivity 0.01g)
- Single-channel micropipette (20 μL-200 μL,100 μL-1000 μL)
- 300 μL multichannel micropipette
- Distilled or deionized water

ⓘNotes:

- Before using the kit, read the instructions carefully.
- Do not use expired kits, and do not mix reagents in kits with different batch numbers.
- Kindly use graduated containers to prepare the reagent.
- Bring all reagents to room temperature (20-25°C) before use for 30 min.
- Only the disposable tips can be used for the experiments and the tips must be changed when used for different reagents.
- Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.
- The stop solution is acidic. Wear eyes, hands, face, and clothing protection when using the product.
- EnkiLife is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
- If the samples are not indicated in the manual, a preliminary experiment to determine

the validity of the kit is necessary.

- Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

Reagent Preparation

- **Wash Buffer (1x):** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 10 mL of Wash Buffer (20x) into 190 mL deionized or distilled water to prepare 200 mL of Wash Buffer (1x). Keep it at 4 °C for one month.

Sample Preparation and collection

The prepared sample may be stored for up to one day at 4°C.

Raw milk, sterilized milk, pasteurized milk, modified milk: Take an appropriate amount of the sample into a 15 mL centrifuge tube and centrifuge at a low temperature (4-10℃) above 4000 rpm 10 min. Avoid the upper layer fat and take 100 μL of the middle layer for analysis. Dilution factor of sample: 1.

Yoghurt: Weigh 1±0.05 g of fresh milk sample into a 10 mL centrifuge tube, add 400 μL of **Yoghurt extract**, 2.6 mL of deionized water, and vortex thoroughly for 1 min. Centrifuge at 4000 rpm for 10 min at 4-10 ℃. Avoid the upper layer fat and take 100 μL of the middle layer for analysis. Dilution factor of sample: 4.

Milk powder, whey powder, whey protein powder: Weigh 1±0.05 g of the sample into a 10 mL centrifuge tube, add 5 mL of deionized water, and Vortex thoroughly until completely dissolved. Centrifuge at 4000 rpm for 10 min at 4-10 ℃. Avoid the upper layer fat and take 100 μL of the middle layer for analysis. Dilution factor of sample: 6.

Milk drink: Weigh 1±0.05 g of fresh milk sample into a 10 mL centrifuge tube, add 400 μL of **Yoghurt extract**, 600 μL of deionized water, and vortex thoroughly for 1 min. Centrifuge at 4000 rpm for 10 min at 4-10 ℃. Take 100 μL of the supernatant for analysis. Dilution factor of sample: 2.

Assay Protocol

ⓘNotes:

- Take the required reagent out of the refrigerated environment at 4°C and place it at room temperature for more than 30 min. If the reagent is crystalline, allow it to dissolve sufficiently at room temperature. Shake each liquid reagent well before use.
- It is recommended that all standards and samples be run at least in duplicate.
- Remove the required number of assay plates, put the unused assay plates in sealed bags, and store at 4°C.
- Avoid using metal packaging and stirring reagents.
- Mix the liquid well and complete removal of liquid at each step is essential to good performance.
- Discard the substrate with any color that indicates the degeneration of this solution. When the absorbance value of 0ppb standard less than 0.5 indicates its degeneration.
- The sample addition time for each step shall not exceed 3 min.

1、**Number:** Insert the required microplate strips into the microplate rack and record the positions of the negative control, positive control and samples. It is recommended to conduct parallel experiments for all of them.

2、**Add Sample:** Add 100 µL of the negative control, positive control and sample solution to the corresponding wells respectively. Cover the assay plate with a plate sealer, gently shake for 10s to mix well, and react in the dark at 37 °C for 60 min.

3、**Washing:** Carefully remove the plate sealer, discard the liquid in the plate wells. Wash by filling each well with 350 µL of **Wash Buffer (1X)** using a squirt bottle, multi-channel pipette, manifold dispenser, or auto washer, and let it stand for 30s. Aspirate or decant the liquid in the plate wells, pat it dry against clean absorbent paper, and complete one wash. Repeating the process 5 times.

Tips: After the plate wells is dried, the next step should be carried out immediately.

4、**Add Antibody Working Solution：** Add 100 µL of **Antibody** per well. Cover the assay plate with a plate sealer, gently shake for 10s to mix well, and react in the dark at 37 °C for 30 min. Repeat the **Washing** steps after that.

5、**Add HRP Conjugate：** Add 100 µL of **HRP Conjugate** per well. Cover the assay plate with a plate sealer, gently shake for 5s to mix well, and react in the dark at 37 °C for 15 min. Repeat the **Washing** steps after that.

6、**Add Substrate Reagent:** Add 50 µL of **Substrate Reagent A** to each well, then add 50

µL of **Substrate Reagent B**, gently shake for 5s to mix well. React in the dark for 15 min at 37°C.

Tips: Adjust the incubation time according to the color change, but do not exceed 30 min. Once the standard wells show a clear gradient, the incubation can be stopped.

7、**Stop the Reaction:** Add 50 µL of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.

8、**Measure the OD value:** Determine optical density (OD) result at 450 nm within 10 min. (Recommend reading the OD value at the dual-wavelength: 450/630 nm).

Interpretation of the results

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

NC= Average absorbance of negative control.
T =NC+0.2.
Positive result: Average absorbance of sample ≥ T.
Negative result: Average absorbance of sample < T.

We are always committed to providing high-quality products and thank you for your understanding and support. If you have any questions, please feel free to contact our technical support team.