(FOR RESEARCH USE ONLY. DO NOT USE IT IN DIAGNOSIS!)

Cat No.: EKF1023

Sulbactam (SUL) 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 Sulbactam content in Food samples.

Key Features

- Sensitivity：0.5 ppb (ng/mL)
- Detection limits：

Raw milk	50 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 assay employs the competitive inhibition enzyme immunoassay technique. The kit consists of an enzyme-labeled plate pre-coated with coupled antigens, β-Lactamase Lyophilized Microplate, reaction solution, and other supporting reagents. During detection, negative control or sample solutions are added to the β-Lactamase Lyophilized Microplate, and reaction solution A is added to complete the specific reaction. Then transfer the reaction solution to the sulbactam microplate and add reaction solution B. The substrate reagent is added to initiate the color developing reaction. The presence of SUL can be determined according to the OD value by using a microplate reader with 450 nm (630 nm) wavelength.

Materials Supplied

Item	Quantity
Sulbactam ELISA Plate	96T
β-Lactamase Lyophilized Microplate	96 wells
Lyophilized microplate of reaction solution A	24 wells
Concentrated reaction solution B (5×)	1 x 1.5 mL
Diluted reaction solution B	1 x 6 mL
Negative Control	1 x 5 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.
- **Reaction Solution A:** According to the need, take the required amount of **Lyophilized microplate of reaction solution A**. Dissolve one well in 100 μL of deionized water, mix well and transfer all to a 5 mL centrifuge tubes. Then add 800 μL of deionized water and mix well.
- **Reaction Solution B:** Take 100 μL of the **Concentrated reaction solution B (5×)** and add it to a 2 mL centrifuge tube, then add 400 μL of the **Diluted reaction solution B** and mix well.

Sample Preparation and collection

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

Raw milk: If the fat content of milk is too high, the milk can be centrifuged 4000 rpm for 5 min and then detected. Take 100 μL of the well-mixed raw milk for analysis. Dilution factor of sample: 1.

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、According to the need, take the required amount of **β-Lactamase Lyophilized Microplate**. Add 50 μL of deionized water to each well, then add 100 μL of **Negative Control** and **Sample solution** to the corresponding wells respectively, gently blow and stir 10 times to mix well. Cover the assay plate with a plate sealer, and react in the dark at 25 °C for 15 min.

2、Carefully remove the plate sealer, add 50 μL of **Reaction solution A** to each well. Cover the assay plate with a plate sealer, gently shake for 20s to mix well, and react in the dark at 25 °C for 15 min.

3、Carefully remove the plate sealer, take 100 μL from each well respectively and add it to the **Sulbactam ELISA Plate.** add 50 μL of **Reaction solution B** to each well. Cover the assay plate with a plate sealer, gently shake for 20s to mix well, and react in the dark at 25 °C for 30 min.

4、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.

5、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 25°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.

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

7、**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 positive control > 0.5.

NC= Average absorbance of negative control ÷ Average absorbance of sample.
Positive result: NC ≤ 0.5.
Negative result: NC > 0.5.

ℹ 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.