β-lactams (Beta-lactam) Antibiotic ELISA Kit

Catalog No: E-FS-E065

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

Version Number:	V1.2
Replace version:	V1.1
Revision Date:	2026.01.04

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 Competitive-ELISA as the method for the quantitative detection. It can detect Beta-lactam Antibiotic (β-lactams) in muscle and other samples. This kit is composed of ELISA Microtiter plate pre-coated with coupled antigen, HRP conjugate, antibody, standard and other supplementary reagents. During the detection, after adding standard or sample solution, β-lactams in the samples competes with pre-coated coupled antigen on the ELISA Microtiter plate for β-lactams antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each well, and Substrate Reagent is added for color development. There is a negative correlation between the OD value of samples and the concentration of β-lactams. The concentration of β-lactams in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Reaction mode (Incubation time and temperature): 25±2℃; 30 min, 30 min, 15-20 min.

Detection limit: Muscle---10 ppb; Raw milk, Milk, Reconstituted milk---1 ppb; Eggs---2 ppb.

Cross-reactivity: Cephacetrile---5%; Cephazoline---5%; Nafricillin---30%; Cefotaxime Sodium---30% Cefapirin---30%; Amoxicillin--100%; Ampicillin---110%; Cloxacillin---110%; Oxacillin---85%; Penicillin---500%; Dicloxacillin---210%; Cefoperazone---250%; Cephalonium---400%.

Sample recovery rate: 90%±30%.

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1.5 mL each (ppb=ng/mL=ng/g) (0 ppb, 0.2 ppb, 0.6 ppb, 1.8 ppb, 5.4 ppb, 16.2 ppb)
HRP Conjugate	12 mL
Antibody Working Solution	7 mL
Substrate Reagent A	7 mL
Substrate Reagent B	7 mL
Stop Solution	7 mL
Milk Extractant	50 mL
Milk Diluent	10 mL
Egg Diluent	50 mL
20×Concentrated Wash Buffer	25 mL
5×Concentrated Sample Diluent	20 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

Instruments: Microplate Reader, Homogenizer, Vortex mixer, Centrifuge, Balance (sensibility 0.01 g).
Micropipette: Single channel (20-200 μL, 100-1000 μL), Multichannel (30-300 μL).
Reagents: Acetonitrile (AR Grade), Methanol.

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-E065. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E065 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

Bring 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**

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

   Solution 1: **70% Methanol Solution**

   Dissolve 350 mL of **Methanol** with 150 mL deionized water, mix fully.

   Solution 2: **Sample Diluent**

   Dilute the **5×Concentrated Sample Diluent** with deionized water. (5×Concentrated Sample Diluent (V): Deionized water (V) =1:4).

   Solution 3: **Wash Buffer**

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

4. **Sample pretreatment procedure**

3.1 Pretreatment of muscle (chicken, pork, mutton, beef, duck, fish, shrimp) sample:

(1) Remove fat from sample, homogenize the sample with homogenizer.

(2) Weigh 2±0.05 g of homogenate sample into 50 mL centrifuge tube, add 4 mL of **70% Methanol Solution** (Solution 1), vortex for 1 min.

(3) Centrifuge at 4000 r/min for 5 min at room temperature.

(4) Take 50 µL of supernatant to another centrifuge tube, and add 450 µL of **Sample Diluent** (Solution 2), vortex for 10 s.

(5) Take 50 µL for analysis.

Note: Sample dilution factor: 20, detection limit: 10 ppb

3.2 Pretreatment of raw milk, milk, reconstituted milk sample:

Reconstituted milk: Weigh 1.00 ± 0.05 g of milk powder into a 10 mL centrifuge tube, add 5 mL of deionized water, and completely dissolve it for later use.

(1) Take 1 mL of sample to centrifuge tube, add 0.5 mL of **Milk Extractant**, vortex for 1 min.

(2) Centrifuge at 4000 r/min for 5 min at room temperature.

(3) Take 300 µL of middle layer to another centrifuge tube, and add 100 µL of **Milk Diluent,** vortex for 10 s.

(4) Take 50 µL for analysis.

Note: Sample dilution factor: 2, detection limit: 1 ppb

3.3 Pretreatment for eggs sample:

(1) Homogenate the eggs sample with homogenizer to mix fully.

(2) Weigh 2±0.05 g of homogenate sample into 15 mL centrifuge tube, add 2 mL of **Acetonitrile**, vortex for 1 min.

(3) Centrifuge at 4000 r/min for 5 min at room temperature.

(4) Take 0.5 mL of the upper layer liquid and dry at 60-70℃ with nitrogen evaporators or water bath (Please do it in a ventilated environment).

(5) Add 0.5 mL of **Egg Diluent** to dissolve the remaining dry material, vortex for 30 s.

(6) Take 50 µL for analysis.

Note: Sample dilution factor: 1, detection limit: 2 ppb

Assay procedure

Restore all reagents and samples to room temperature (25±2℃) 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. **Standard and Samples need test in duplicate**.
2. **Add Sample:** add 50 µL of **Standard** or **Sample** per well, then add 50 µL of **Antibody Working Solution** into each well, cover the plate with plate sealer. Oscillate for 10 s gently to mix thoroughly, incubate for 30 min at 25±2℃ in shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid of each well. Immediately add 260 µL of **Wash Buffer** (Solution 3). Repeat wash procedure for 4 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).
4. **HRP Conjugation:** add 100 µL of **HRP Conjugate** into each well, and incubate at 25±2℃ for 30 min.
5. **Wash:** repeat step 3 for washing.
6. **Color Development:** add 100 µL mixture of **Substrate Reagent A** and **Substrate Reagent B** to each well (**Substrate Reagent A** and **Substrate Reagent B** are fully mixed at ratio of 1:1 according to 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 for 10-15 min at 25±2℃ in the dark (The reaction time can be extended according to the actual color change).
7. **Stop Reaction:** add 50 µL of **Stop Solution** to each well. Gently oscillate for 10 s to mix thoroughly
8. **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.

Result analysis

1. **Absorbance% =A/A_0×100%**

   A: Average absorbance of standard solution or sample

   A_0: Average absorbance of 0 ppb Standard solution

2. **Drawing and calculation of standard curve**

   Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add the average absorbance value to standard curve to get corresponding concentration. **If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.**

   For this kit, it is more convenient to use professional analysis software for accurate and fast analysis on a large number of samples.

Beta-lactam Antibiotic (E-FS-E065) **Standard Curve**