

BAC (Bacitracin) ELISA Kit

Catalog No: E-FS-E141

96T/96T*3

Version Number:	V1.3
Replace version:	V1.2
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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.

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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 Bacitracin (BAC) in samples, such as milk, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, standard and other supplementary reagents. The microtiter plate provided in this kit has been pre-coated with coupled antigen. During the detection, BAC in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-BAC 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 BAC. The concentration of BAC 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°C; 45 min, 15-20 min

Detection limit: Milk, Muscle, Egg---100 ppb

Cross-reactivity: Bacitracin ---100%

Sample recovery rate: 100%±30%.

Kits components

Item	Specifications
ELISA Microtiter Plate	96 wells
Standard Liquid	1.5 mL each (ppb=ng/mL=ng/g) (0 ppb, 2 ppb, 6 ppb, 18 ppb, 54 ppb, 162 ppb)
Antibody Working Solution	7 mL
HRP Conjugate	7 mL
Substrate Reagent A	7 mL
Substrate Reagent B	7 mL
Stop Solution	7 mL
Sample Diluent A	50 mL
Sample Diluent B	50 mL
20×Concentrated Wash Buffer	25 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

Instrument: Microplate reader, Printer, Nitrogen evaporators, Water bath, Oscillators, Centrifuge, Graduated pipette, Balance (sensitivity 0.01 g).

Micropipette: Single channel (20-200 μL , 100-1000 μL), Multichannel (30-300 μL).

Reagents: Anhydrous 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 $25\pm 2^\circ\text{C}$.
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. 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-E141. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E141 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 ($A_{450\text{nm}} < 0.8$), 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. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
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 $2-8^\circ\text{C}$. 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 $2-8^\circ\text{C}$. After opening, the kit is stable for up to 1 month.

Expiry date: expiration date is on the packing box.

Experimental preparation

Restore 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

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 **20×Concentrated Wash Buffer** with deionized water (20×Concentrated Wash Buffer (V): Deionized water (V) = 1:19), mix fully.

3. Sample pretreatment procedure

3.1 Pretreatment of milk sample:

- (1) Take 1 mL of fresh sample into a 10 mL centrifuge tube, then add 3 mL of **Anhydrous methanol**, oscillate for 3 min.
- (2) Centrifuge for 10 min at 4000 g/min.
- (3) Take 50 µL of the supernatant into a 2 mL centrifuge tube. Add 0.45 mL of **Sample Diluent A** and fully oscillate for 30 s.
- (4) Take 50 µL for analysis.

Note: Sample dilution factor: 40, detection limit: 100 ppb

3.2 Pretreatment of muscle (livestock, poultry), egg sample:

- (1) Remove fat from sample. Homogenize the representative sample with a homogenizer and mix fully.
- (2) Take 1 ±0.05g of homogeneous sample into a 10 mL centrifuge tube, then add 1 mL of deionized water and 3 mL of **anhydrous methanol** to fully oscillate for 3 min.
- (3) Centrifuge for 10 min at 4000 g/min.
- (4) Take 50 µL of the supernatant into a 2 mL centrifuge tube. Add 0.45 mL of **Sample Diluent B** to fully oscillate for 30 s.
- (5) Take 50 µL for analysis.

Note: Sample dilution factor: 50, detection limit: 100 ppb

Assay procedure

Restore all reagents and samples to room temperature 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°C.

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, Add 50 µL of **HRP Conjugate**, then add 50 µL of **Antibody Working Solution** into each well. Gently oscillate for 10 s to mix thoroughly and cover the plate with plate sealer. Incubate at 25±2°C for 45 min in shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 260 µL of **Wash Buffer** (Solution 1) to each well and wash. Repeat the wash procedure for 4 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. **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 15-20 min at 25±2°C in the dark (The reaction time can be extended according to the actual color change).
5. **Stop Reaction:** add 50 µL of **Stop Solution** to each well, oscillate gently for 10 s to mix thoroughly.
6. **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(%)= $A/A_0 \times 100\%$

A: Average absorbance of standard or sample

A_0 : Average absorbance of 0 ppb Standard

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 average absorbance value of sample 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 form for accurate and fast analysis of batch samples.

Bacitracin (E-FS-E141) Standard Curve

