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User's Manual

Bacitracin ELISA kit

REF **DEIA-XY24**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Bacitracin ELISA Test Kit is a competitive immunoassay to quantitatively detect the presence of BAC in shrimp, pork, beef, chicken, egg, and milk samples.

General Description

Bacitracin (BAC) is a polypeptide antibiotic. In addition to veterinary applications, BAC can be added to feed and used as an animal growth promoter. Whenever these drugs are administered to food producing animals, residues in milk, eggs or edible tissues are likely. These residues can cause allergic reactions but also promote antibiotic resistance of bacteria which may pose a health risk for the population.

Principles of Testing

This test kit is based on a competitive enzyme-linked immunosorbent assay (ELISA) for the detection of BAC. An unknown amount of BAC present in the sample and the fixed amount of BAC antigens pre-coated onto the wells of microtiter plate/strips compete for the anti-BAC antibodies, which in turn are detected with enzyme conjugate. After incubation, the wells are washed and the bound enzyme is visualized by adding TMB solution. Any coloured product is measured at 450 nm after adding stop solution. The absorbance value of the developed colour is inversely proportional to the amount of BAC in the sample. The quantity of BAC in the test sample can be calculated using the standard curve constructed from the standards, and corrected for the sample dilution.

Reagents And Materials Provided

1. Pre-coated microtitre plate, 12 x 8 wells
2. BAC antibody solution, 1 x 7 ml
3. Enzyme conjugate, 1 x 7 ml
4. Wash buffer (20x concentrate), 1 x 30 ml
5. Assay diluent (5x concentrate), 1 x 30 ml
6. Tissue Sample Preparation Reagent, 2 x 50 ml
7. BAC standards, (0, 3, 9, 27,81, 243 ppb), 0.5 ml each
8. High concentrate of BAC standard, (1000 ppb)* 1 x 1 ml
9. TMB solution (ready to use) 1 x 12 ml
10. Stop solution (ready to use) 1 x 12 ml
11. Microplate sealer 1 piece
12. Package insert 1 copy

* This component is optional and only for the user to check the recovery rate of BAC.

Materials Required But Not Supplied

1. ELISA Microtiter plate reader equipped with 450/630 nm filters
2. Single and multichannel micropipettes from 50 µl to 1.0 ml, with disposable pipette tips
3. Plate shaking equipment
4. Microplate washer or squeeze bottle
5. Centrifuge
6. Vortexer
7. Centrifugal tubes
8. Eppendorf tubes
9. Deionised water (ddH_2O)
10. Absorbent pads (tissue)

Storage

• The kit should be stored at 2–8°C. Do not freeze.

• Unused test wells should be sealed and stored at 2–8°C.

• This kit is valid until the expiration date printed on the label.

Specimen Collection And Preparation

Shrimp, Pork, Beef & Chicken (Dilution factor: 30)

• Slice sample and weigh 1 g into a centrifugal tube.

• Add 2 ml of tissue sample preparation reagent and mix well.

• Centrifuge the sample at 4,000 x g for 10 mins.

• Transfer 100 µl of the supernatant to an eppendorf tube and add 900 µl of diluted assay diluent. Mix well.

• Use 50 µl as a sample in the assay.

Egg (Dilution factor: 30)

• Weigh 1 g of homogenised sample into a centrifugal tube.

• Add 2 ml of tissue sample preparation reagent and mix well.

• Centrifuge the sample at 4,000 x g for 10 mins.

• Transfer 100 µl of the supernatant to an eppendorf tube and add 900 µl of diluted assay diluent. Mix well.

• Use 50 µl as a sample in the assay.

Milk (Dilution factor: 10)

• Measure 1 ml of milk into an eppendorf tube.

• Centrifuge the sample at 10,000 x g for 10 mins.

• Transfer 100 µl of the supernatant to an eppendorf tube and add 900 µl of diluted assay diluent. Mix well.

• Use 50 µl as a sample in the assay.

Samples should be tested as soon as possible after preparation.

Reagent Preparation

Wash buffer: Dilute the wash buffer concentrate (20x) 20-fold with deionised water (e.g. 10 ml wash buffer concentrate with 190 ml ddH_2O).

Assay diluent: Dilute the assay diluent concentrate (5x) 5-fold with deionised water (e.g. 20 ml assay diluent concentrate with 80 ml H_2O).

Note: Wash buffer concentrate and assay diluent concentrate may form crystals at low temperature. Ensure that the crystals completely re-dissolve before dilution (by placing into a 37°C incubator or water bath if necessary)

Assay Procedure

1. Ensure all reagents are equilibrated to RT prior to use. Swirl all reagents gently before use.
2. Label each strip on its end tab to help identify them should they become detached from the plate frame during the assay. Surplus wells should be placed back into the re-sealable foil pouch with desiccant and stored at 2 - 8°C
3. To every well (except the two blank wells) add 50 µl of each standard solution or sample solution per well in duplicate, then add 50 µl of enzyme conjugate to each well, finally add 50 µl antibody solution to each well. (Note: This order of addition is very important)
4. To the two blank wells, add 100 µl of diluted assay diluent and 50 µl enzyme conjugate. (No standards/samples or antibody solution)
5. Cover the strips with the microplate sealer and shake gently to mix for 1 minute. Incubate the plate for 30 minutes at 37°C in the dark.
6. After incubation, remove the plate sealer and wash the strips 5 times with diluted wash buffer, ensuring every well is filled. When washing is completed, tap the strips firmly on absorbent tissue to remove residual wash buffer.
7. Add 100 µl of the TMB solution to each well and incubate for 10 minutes at 37°C in the dark.
8. Stop the reaction by adding 100 µl of stop solution to each well in the same order as the TMB solution was added. Shake gently to mix.
9. Measure absorbance at 450 nm (with 630 nm as a reference filter) within 10 minutes of stopping

Quality Control

For the test to be valid, the mean absorbance of the zero standard (S1, 0 ppb) must be over 1.0

Calculation

The unknown BAC concentrations in the samples are determined from a standard curve. Calculate the mean absorbance value of the two blank wells and subtract that from the mean absorbance values of all the other wells.

Define the mean corrected absorbance value of the standards and samples as B. Define the mean corrected absorbance of the zero standard as B0. The relative absorbance can therefore be calculated as:

Relative absorbance (%) = B/B0×100%

Plot the relative absorbance of the standards against the standard concentration to obtain a standard curve. Using the relative absorbance value of a sample, the concentration can be found by interpolation.

Remember to multiply by the dilution factor to obtain the true BAC concentration.

Interpolation can be performed by carrying out a 4-parameter logistic analysis, using a linear regression method, or point-to-point interpolation.

Notes

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in this package insert and with adherence to good laboratory practice.
2. Factors that might affect the performance of the assay include proper instrument function/calibration, cleanliness of glassware, quality of distilled or deionised water, accuracy of reagent and sample pipetting, washing technique, incubation time and temperature

Detection Limit

Shrimp sample: 90 ppb

Pork sample: 90 ppb

Beef sample: 90 ppb

Chicken sample: 90 ppb

Egg sample: 90 ppb

Milk samples: 30 ppb

Specificity

Bacitracin: 100%

Kanamycin: <0.1%

Neomycin :< 0.1%

Gentamicin :< 0.1%

Recovery

Shrimp sample: 70-110%

Pork sample: 70-110%

Beef sample: 70-110%

Chicken sample: 70-110%

Egg samples: 75-110%

Milk samples: 70-120%

Precautions

1. Please carefully read the instructions before use.
2. Reagents should be brought to room temperature (RT, 18-25°C) prior to use.
3. Do not use reagents after the expiration date. Do not use reagents from other kits with different lot numbers.
4. Avoid contact of skin and mucous membranes with reagents and sample extraction. If exposure should occur, immediately flush with water.
5. Please wear protective gloves when using the kit. Consider all materials that are exposed to standards or samples to be contaminated.
6. Use different tips when pipetting different reagents and samples.
7. Keep the stop solution away from skin and eyes.