Beta-Lactam Antibiotic (B-Lactams) ELISA Kit

Catalog No.: abx365001

Size: 96T

Storage: Store at 4 °C. Once opened, store at 4°C and use within 1 month.

Application: For quantitative detection of B-Lactam in muscle, egg and milk samples.

Sensitivity: 0.2 ng/ml (ppb)

Detection Limit: Milk – 1 ng/ml; Muscle, Eggs – 10 ng/ml

Cross-Reactivity: Amoxicillin – 100%; Penicillin – 100%; Cephacetrile – 5%; Dicloxacillin – 100%; Ampicillin – 110%; Oxacillin – 85%; Cephazoline – 5%; Nafricillin – 30%, Cefotaxime Sodium – 30%; Cefapirin – 30%; Cloxacillin – 110%; Cefoperazone – 100%; Cephalonium – 100%

Introduction: Beta-lactams, a class of antibiotics identified by the presence of a lactam ring within their chemical structure, are widely used to treat many bacterial infections in humans and animals, including animals for human consumption. It functions similarly to penicillin by inhibiting cell wall synthesis, resulting in bacterial cell lysis.

Principle of the Assay

This kit is based on the competitive enzyme-linked immuno-sorbent assay technology. This kit is composed of a 96-well plate pre-coated with the coupled antigen, HRP conjugate, antibody, standard and other supplementary reagents. During the detection, after adding the standard or sample solution, Beta-lactams in the sample competes with the fixed pre-coupled antigen for the antibody against Beta-lactams. The HRP conjugate is then added, followed by the substrate reagent for colour development. The intensity of the yellow color is inversely proportional to the levels of Beta-lactams bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, from which the concentration of Beta-lactams can be calculated.

Kit components

1. One pre-coated 96 well plate
2. Concentrated Wash Buffer (20X): 25 ml
3. Standard (0 ppb, 0.2 ppb, 0.6 ppb, 1.8 ppb, 5.4 ppb,16.2 ppb): 1 ml each
4. Sample Diluent (5X): 20 ml
5. Detection Reagent A: 7 ml
6. Detection Reagent B: 12 ml
7. Substrate Reagent A: 6 ml
8. Substrate Reagent B: 6 ml
9. Stop Solution: 6 ml
10. Milk Extractant: 50 ml
11. Milk Diluent: 10 ml
12. Plate Sealer: 3
13. Hermetic Bag

Materials Required But Not Provided

1. Microplate reader (450 nm) and incubator
2. High-precision pipette and sterile pipette tips
3. Automated plate washer (optional)
4. ELISA shaker (optional)
5. Centrifuge and 50 ml centrifuge tubes
6. Absorbent filter papers
7. Homogenizer
8. Scale (precision 0.01 g)

Reagents Required But Not Provided

1. Deionized water
2. Methanol

Protocol

A. Preparation of sample and reagents

1. Preparation of reagents

- **70% Methanol Solution**
  Dilute 35 ml of methanol with 15 ml of deionized water to make the 70% methanol solution. Mix thoroughly.
- **Wash Buffer Solution**
  Dilute the 20 X Concentrated Wash Buffer 20-fold with deionized water (i.e. dilute 25 ml 20X Concentrated Wash Buffer in 475 ml deionized water) to make the 1X Wash Buffer solution.
- **Sample Diluent Solution**
  Dilute the 5 x Concentrated Sample Diluent 5-fold with deionized water (i.e dilute 20 ml of Concentrated Sample Diluent with 80 ml deionized water) to make the 1 X Sample Diluent Solution.

2. Sample pretreatment

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

- **Milk:** Take 1 ml of fresh sample and add to a centrifuge tube. Add 0.5 ml of Milk Extractant. Vortex for 1 minute, then centrifuge at 4000 RPM for 5 minutes. Take 300 µl of the middle layer and add to another centrifuge tube. Add 100 µl of Milk Diluent. Vortex for 1 minute, then take 50 µl for analysis.
  Note: Sample dilution factor: 2, minimum detection dose: 1 ng/ml.
- **Muscle and Egg:** Remove fat and homogenize samples. Weigh 2 ± 0.05 g of sample and add to a centrifuge tube. Add 4 ml of 70% Methanol Solution. Vortex for 1 minute, then centrifuge at 4000 RPM for 5 min at room temperature. Aliquot 50 µl of the supernatant and add to another centrifuge tube with 450 µl of Sample Diluent Solution. Vortex for 1 minute, then take 50 µl of the supernatant for analysis.
  Note: Sample dilution factor: 20, minimum detection dose: 10 ng/ml

Notes:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C.
- Samples should be clear and transparent. Samples must be diluted so that the expected concentration falls within the kit's range.
- Please bring samples slowly to room temperature. Samples that contain NaN_3 cannot be detected as it interferes with HRP.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

1. Set standard and sample wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate.
2. Add 50 µl of the standard solutions into the standard wells. Add the solution at the bottom of each well without touching the side wall.
3. Add 50 µl of prepared sample into the sample wells.
4. Add 50 µl of Detection Reagent A to each well.
5. Cover the plate with the plate sealer. Tap the plate gently to ensure thorough mixing. Incubate at 25°C for 30 minutes in dark conditions.
6. Remove the cover, and discard the solution without touching the side walls. Blot the plate on an absorbent material. Add 260 µl of wash buffer to each well and soak for at least 30 seconds. Discard the contents and blot the plate on absorbent material. Repeat this procedure for a total of five times.

Note: For automated washing, discard the solution in all wells and wash five times overfilling the wells with Wash buffer. After the final wash invert the plate and tap on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 minute.

7. Add 100 µl of Detection Reagent B into each well. Add the solution at the bottom of each well without touching the side walls.
8. Cover the plate with the plate sealer. Tap the plate gently to ensure thorough mixing. Incubate in the dark at 25°C for 30 minutes.
9. Repeat the wash step as described in step 6.
10. Add 50 µl of Substrate Reagent A and 50 µl of Substrate Reagent B into each well. Tap the plate gently to ensure thorough mixing. Cover the plate and incubate at 25°C in dark conditions for 15-20 minutes (incubation time is for reference only, do not exceed 30 minutes). When an apparent gradient appears in the standard wells the reaction can be terminated.
11. Add 50 µl of Stop solution into each well (including the blank well). There should be a color change to yellow. Gently tap the plate to ensure thorough mixing.
12. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.
13. OPTIONAL: measure the absorbance at 630 nm and subtract these values from the 450 nm values for analysis.

This assay is competitive, therefore there is an inverse correlation between Beta-lactams concentration in the sample and the absorbance measured. Create a graph with the log of the standard concentration (y-axis) and absorbance measured (x-axis). Apply a best fit trendline through the standard points. Use this graph calculate sample concentrations based on their OD values. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.

C. Precautions

1. Equilibrate all reagents to room temperature (18-25°C) prior to use and centrifuge the tubes briefly in case any contents are trapped in the lid.
2. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.
3. Wash buffer may crystallize and separate. If this happens, please warm the tube and mix gently to dissolve.
4. Avoid foaming or bubbles when mixing or reconstituting components.
5. It is recommended to assay all standards, controls and samples in duplicate or triplicate.
6. Do NOT let the plate dry out completely during the assay as this will inactivate the biological material on the plate.
7. Ensure plates are properly sealed or covered during incubation steps.
8. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
9. To avoid cross contamination do not reuse pipette tips and tubes.
10. Do not use components from a different kit or expired ones.
11. Substrate Reagent A and Substrate Reagent B are easily contaminated; work under sterile conditions when handling these solutions. The substrate solutions should also be protected from light. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not replace the residual solution back into the vial.