Amoxicillin ELISA Kit

Catalog No.: abx364995

Size: 96T

Storage: Store at 4°C.

Application: For quantitative detection of Amoxicillin in muscle, egg and milk samples.

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

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

Sample Recovery Rate: 90% ± 30%

Introduction: Amoxicillin is a beta-lactam antibiotic which is 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 competitive enzyme-linked immuno-sorbent assay technology. Amoxicillin is pre-coated onto a 96-well plate. The standards and samples and a biotin conjugated antibody specific to Amoxicillin are added to the wells and incubated. After washing away the unbound conjugates, avidin conjugated to Horseradish Peroxidase is added to each microplate well. After TMB substrate solution is added only wells that contain Amoxicillin will produce a blue color product that changes into yellow after adding the stop solution. The intensity of the yellow color is inversely proportional to the Amoxicillin amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of Amoxicillin can be calculated.

Kit Components

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

Materials Required But Not Provided

1. Microplate reader (450 nm) and incubator
2. High-precision pipette and sterile pipette tips
3. Squirt bottle or automated plate washer
4. ELISA shaker
5. 50 ml centrifuge tubes
6. Absorbent filter papers
7. 100 ml and 1 L graduated cylinders
8. Homogenizer
9. Deionized water
10. Methanol (70% or 100%)

Protocol

A. Preparation of sample and reagents

1. Preparation of reagents

- **1X Wash Buffer**
  Dilute the 20X Wash Buffer 20-fold with deionized water to prepare the 1X Wash Buffer (e.g. dilute 25 ml 20X Wash Buffer in 475 ml deionized water to make 500 ml of 1X Wash Buffer).

- **1X Sample Solution (for muscle and egg samples)**
  Dilute the 5X Sample Solution 5-fold with deionized water to prepare the 1X Sample Solution (e.g. dilute 20 ml 5X Sample Solution in 80 ml deionized water to make the 100 ml of 1X Sample Solution).

- **70% Methanol (for muscle and egg samples)**
  Dilute 100% Methanol with deionized water to a ratio of 7:3 to prepare the 70% Methanol.

2. Sample pretreatment

- **Milk:** Add 1 ml of fresh milk to a centrifuge tube. Add 0.5 ml of Milk Sample Extraction Solution. Vortex for 1 minute, then centrifuge at 4000 RPM for 5 minutes. Discard the upper fat layer, then aliquot 300 µl of the intermediate layer to another centrifuge tube. Add 100 µl of Milk Diluent and mix thoroughly. Aliquot 50 µl for analysis.
  Note: Sample dilution factor: 2, detection limit: 1 ng/ml.

- **Muscle and Egg:** Remove fat from the sample (except egg samples). Homogenize samples. Weigh 2 g of sample and add to a centrifuge tube. Add 4 ml of 70% Methanol. Vortex for 1 minute, then centrifuge at 4000 RPM for 5 min. Aliquot 50 µl of the supernatant to another centrifuge tube. Add 450 µl of 1X Sample Solution, then vortex for 10 seconds. Aliquot 50 µl for analysis.
  Note: Sample dilution factor: 20, detection limit, 10 ng/ml.

Note:

» 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. *Add solutions to the bottom of each well without touching the side walls. Pipette the standards and samples up and down to mix before adding to the wells. Avoid foaming or bubbles.*

2. Aliquot 50 µl of the standard solutions into the standard wells.

3. Aliquot 50 µl of the standard solutions into the standard wells.

4. Aliquot 50 µl of prepared sample (see Sample pretreatment section above) into the sample wells.

5. Aliquot 50 µl of Detection Reagent A to each well. Cover the plate with a plate sealer. Gently tap the plate or use a microplate shaker to mix. Incubate at 25°C for 30 minutes in dark conditions.

6. Remove the cover and discard the solution. Wash the plate 5 times with 1X Wash Buffer. *Fill each well completely with Wash buffer (260 µl) using a multi-channel Pipette or autowasher (1-2 mins soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.*

7. Aliquot 100 µl of Detection Reagent B to each well. Cover the plate with a plate sealer. Gently tap the plate or use a microplate shaker to mix. Incubate at 25°C for 30 minutes in dark conditions.
8. Remove the cover, discard the solution and repeat the wash process as described above, 5 times
9. Aliquot 50 µl of TMB Substrate A and 50 µl of TMB Substrate B into each well. Cover the plate with a plate sealer. Gently tap the plate to mix thoroughly. Incubate at 25°C in dark conditions for 15-20 minutes. The incubation time is for reference only, the optimal time should be determined by the end user. When an apparent gradient appears in the standard wells the reaction can be terminated.
10. Aliquot 50 µl of Stop Solution into each well. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
11. 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.
12. 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 Amoxicillin 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 to 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. Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not exceed 10 mins.
3. Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled.
4. To avoid cross contamination, do not reuse pipette tips and tubes.
5. Do not use expired components, or components from a different kit.
6. The wash buffer may crystallize and separate. If this happens, warm the tube and mix gently to dissolve the crystals.
7. Avoid foaming or bubbles when mixing or reconstituting components.
8. It is recommended to assay all standards, controls and samples in duplicate or triplicate.
9. Do NOT let the plate dry out completely during the assay as this will inactivate the biological material on the plate.
10. Ensure plates are properly sealed or covered during incubation steps.
11. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
12. TMB Substrate A and TMB Substrate 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.