Ciprofloxacin ELISA Kit

Catalog No.: abx364838

Size: 96T

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

Application: For quantitative detection of ciprofloxacin in eggs, honey, milk, milk powder, and muscle tissue.

Sensitivity: 0.1 ppb (ng/ml)

Detection Limit: Eggs – 3 ppb, Honey – 0.3 ppb, Milk – 2 ppb, Milk Powder – 5 ppb, Muscle Tissue – 0.3 ppb

Introduction: Ciprofloxacin (known under various brand names, including Ciloxan and Neofloxin) is a member of the quinolone family of antibiotics. It inhibits type II and type IV bacterial topoisomerases, preventing cell replication. This antibiotic is typically used to treat a wide variety of bacterial infections, particularly subcutaneous and deep tissue infection, however is no longer frequently administered for respiratory or sexually transmitted infections. Adverse effects can include tendon rupture, cardiotoxicity, and neuropathy.

Principle of the Assay

This kit is based on competitive enzyme-linked immuno-sorbent assay technology. Ciprofloxacin is pre-coated onto a 96-well plate. The standards and samples and a biotin conjugated antibody specific to ciprofloxacin 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 ciprofloxacin will produce a blue colour product that changes into yellow after adding the stop solution. The intensity of the yellow colour is inversely proportional to the ciprofloxacin amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of ciprofloxacin can be calculated.

Kit components

1. One pre-coated 96 well plate
2. Standards - 0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb: 1 ml each
3. Detection Reagent A: 5.5 ml
4. Detection Reagent B: 5.5 ml
5. TMB Substrate A: 6 ml
6. TMB Substrate B: 6 ml
7. Stop solution: 6 ml
8. Wash buffer (20X): 40 ml
9. Reconstitution buffer (5X): 50 ml
10. Plate sealer: 3 pieces
11. Hermetic bag: 1

Materials Required But Not Provided

1. Incubator
2. Microplate reader (450 nm)
3. High-precision pipette and sterile pipette tips
4. Automated plate washer (optional)
5. ELISA shaker (optional)
6. Centrifuge and microfuge tubes
7. Scale (precision 0.01 g)
8. Nitrogen evaporator or water bath
9. Vortex mixer
10. Homogenizer

Reagents Required But Not Provided

1. 0.15 M hydrochloric acid
2. Acetonitrile
3. Deionized water
4. N-hexane
5. Dichloromethane

Protocol

A. Preparation of sample and reagents

1. Preparation of sample pretreatment solutions

- **Sample extraction solution**
  Add 10 ml of 0.15 M hydrochloric acid to 90 ml acetonitrile and mix fully.

- **Reconstitution buffer (1X)**
  Dilute the 5× reconstitution buffer 5-fold with deionized water (i.e. dilute 50 ml 5× reconstitution buffer in 200 ml deionized water) to make the 1× reconstitution buffer solution. The 1× solution can be stored at 4°C for up to one month.

- **Wash buffer (1X)**
  Dilute the 20× Wash Buffer 20-fold with deionized water (i.e. dilute 40 ml 20× Reconstitution buffer in 760 ml deionized water) to make the 1× wash buffer 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.

- **Eggs:** Homogenize egg (albumin and yolk) sample in a tissue homogenizer. Weigh 1 g of homogenate and add to a 15 ml centrifuge tube. Add 5 ml of deionized water and vortex for 1 minute. Take 100 µl of sample into a microfuge tube and add 400 µl of reconstitution buffer. Vortex for 1 minute, and aliquot 110 µl for analysis.
  Note: Sample dilution factor: 30, minimum detection dose: 3 ppb.

- **Honey:** Weigh 1g of honey and add to a 50 ml centrifuge tube. Add 6 ml of sample extraction buffer and vortex for 5 minutes. Add 3 ml of reconstitution buffer and 11 ml of dichloromethane. Vortex for 5 minutes, then centrifuge at 4000 rpm for 5 minutes. Aspirate and discard the upper phase, and transfer 8 ml of the lower phase to a dry glass tube. Dry in a nitrogen or water bath at 50 - 60°C. Dissolve the residue 1 ml of reconstitution buffer, and add 1ml of n-hexane. Vortex for 30 seconds, then centrifuge at 3000 rpm for 5 minutes. Discard the upper phase, and aliquot 110 µl of the lower phase and assay immediately.
  Note: Sample dilution factor: 2, minimum detection dose: 0.3 ppb.

- **Milk:** Add 475 µl of 1x reconstitution buffer to 25 µl of milk. Vortex for 1 minute, and aliquot 110 µl for analysis.
  Note: Sample dilution factor: 20, minimum detection dose: 2 ppb.

- **Milk powder:** Weigh 0.5 g of milk powder and add 5 ml of water. Vortex to milk, then take 100 µl of sample with 400 µl of reconstitution buffer. Vortex for 1 minute, and aliquot 110 µl for analysis.
  Note: Sample dilution factor: 5, minimum detection dose: 5 ppb.

- **Muscle Tissue:** Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Weigh 2 g of homogenized muscle tissue and add to a 50 ml centrifuge tube. Add 8 ml of sample extraction solution and vortex for 5 minutes. Centrifuge the homogenates at 4000 rpm for 10 min and collect 2 ml of the upper liquid phase. Transfer to a 10 ml glass tube and dry in a nitrogen or water bath at 50 - 60°C. Dissolve the solid residue in 1 ml of n-hexane, and vortex for 2 minutes. Add 1 ml of reconstitution buffer and vortex for 30 seconds to mix. Centrifuge at 4000 rpm for 5 minutes and discard the upper phase. Aliquot 110 µl of the lower phase and assay immediately.
  Note: Sample dilution factor: 2, minimum detection dose: 0.3 ppb.

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 each solution at the bottom of each well without touching the side wall.
3. Add 50 µl of prepared sample into test sample wells.
4. Immediately add 50 µl of Detection Reagent B into each well, and then add 50 µl of Detection Reagent A. Add the solutions at the bottom of each well without touching the side wall.
5. Cover the plate with the plate sealer. Tap the plate gently to ensure thorough mixing. Incubate at 25°C for 45 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 300 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 the wash 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 min.

7. Add 50 µl of TMB Substrate A into each well and then 50 µl of TMB Substrate 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 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.
8. Add 50 µl of Stop solution into each well. There should be a colour change to yellow. Gently tap the plate to ensure thorough mixing.
9. 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.
10. OPTIONAL: measure the absorbance at 630 nm and subtract these values from the 450 nm values for analysis.
11. OPTIONAL: Calculate the absorbance percentage by dividing each OD value by the OD value of the 0 ppb standard, and multiplying by 100 to give a percentage

This assay is competitive, therefore there is an inverse correlation between ciprofloxacin concentration in the sample and the absorbance measured. Create a graph with the log of the standard concentration (x-axis) against absorbance (%) (y-axis). Apply a best fit trend line 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. 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 colourless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

D. Technical Support

For troubleshooting, frequently asked questions, and assistance, please visit: https://www.abbexa.com/scientific-support/troubleshooting-and-faqs/elisa-kit-scientific-support or email us at support@abbexa.com.