Fluoroquinolones (QNs) ELISA Kit

Catalog No.: abx364839

Size: 96T

Storage: Store at 4°C for up to 6 months. For long term storage, the ELISA plate, Standards and Biotin conjugated antibody can be stored at -20°C.

Application: For quantitative detection of QNs in Serum, Tissue, Liver, Honey, Milk, Milk Powder, Egg, Water, Feed and Urine.

Sensitivity: 0.1 ppb (ng/ml)

Detection Limit: Tissue – 0.3 ppb, Honey – 0.4 ppb, Urine – 0.5 ppb, Egg/Milk – 3 ppb, Milk Powder – 6 ppb

Cross-reactivity: Enrofloxacin – 100%, Norfloxacin – 174%, Ciprofloxacin – 170%,Flumequine – 126%, Peflacine – 125%, Danofloxacin – 110%, Sarafloxacin – 107%, Difloxacin – 84%, Enoxacin – 66%, Levofloxacin – 10%, Ofloxacin (racemic) – 58%, Oxolinic acid – 28%, Marbofloxacin – 4%.

Introduction: Fluoroquinolones are broad-spectrum bactericides that share a bicyclic core structure related to the compound 4-quinolone and contain a fluorine atom in their chemical structure. They are effective against both Gram-negative and Gram-positive bacteria. They are often used for genitourinary infections and are widely used in the treatment of hospital-acquired infections associated with urinary catheters.

Principle of the Assay

This kit is based on indirect-competitive enzyme-linked immuno-sorbent assay technology. QNs is pre-coated onto a 96-well plate. The standards and samples and a biotin conjugated antibody specific to QNs 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 QNs 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 QNs amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of QNs can be calculated.

Kit components

1. One pre-coated 96 well plate
2. Standard: 1 ml each of:
   0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb, 100 ppb
3. HRP conjugate reagent: 5.5 ml
4. Antibody solution: 5.5 ml
5. Substrate reagent A: 6 ml
6. Substrate reagent B: 6 ml
7. Stop solution: 6 ml
8. Wash buffer (20X): 40 ml
9. Re-dissolve buffer (5X): 50 ml

Materials Required But Not Provided

1. 37°C incubator
2. Microplate reader (450 nm)
3. High-precision pipette and sterile pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5 ml EP tubes to prepare samples
7. Absorbent filter papers
8. 100 ml and 1 L graduated cylinders

Reagents Required But Not Provided

1. Anhydrous acetonitrile
2. N-hexane
3. Concentrated hydrochloric acid (HCl)
4. Dichloromethane
5. Deionized water

Protocol

A. Preparation of sample and reagents

1. Preparation of Sample pretreatment solutions

- **Solution 1 - 0.15 M Hydrochloric Acid (HCl) Solution**
  Add 5 ml of concentrated HCl to 400 ml of deionized water. Mix thoroughly.

- **Solution 2 – Sample Extraction Solution**
  Add 10 ml of 0.15 M HCl to 90 ml of anhydrous acetonitrile. Mix thoroughly.

- **Solution 3 - Re-dissolve Solution (1X)** *Note: If the sample is aquiform, do not dilute it.*
  Dilute the 5× Re-dissolve Buffer 5-fold with deionized water (i.e. dilute 10 ml 5× Re-dissolve buffer in 40 ml deionized water) to make the 1× Re-dissolve Buffer solution. The 1× solution can be stored at 4°C for up to one month.

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.

- **Tissue:** Weigh 2 ± 0.05 g of homogenate and add to a 50 ml tube. Add 8 ml of Sample Extraction Solution (Solution 2) and mix for 5 min. Centrifuge at 4000 RPM for 10 min at room temperature. Collect 2 ml of supernatant (clear upper organic layer) and dry at 50-60°C with nitrogen or air. Dissolve the residue with 1 ml of n-hexane and mix for 2 min. Add 1 ml of 1× Re-dissolve solution (Solution 3) and mix fully by shaking for 30 sec. Centrifuge at 4000 RPM for 5 min at room temperature. Remove the hexane upper phase and take 50 µl of the lower phase for analysis.
  Note: Sample dilution factor: 2, minimum detection dose: 0.3 ppb.

- **Honey:** Weigh 1 ± 0.05 g of honey and add to a 50 ml tube. Add 6 ml of Sample Extraction Solution (Solution 2) and mix for 5 min. Add 3 ml of 1× Re-dissolve solution (Solution 3) and 11 ml dichloromethane and mix for 5 min. Centrifuge at 4000 RPM for 10 min at room temperature. Collect 8 ml of supernatant (upper organic layer) and dry at 50-60°C with nitrogen or air. Dissolve the residue with 1 ml of 1× Re-dissolve solution (Solution 3) and mix fully by shaking for 30 sec. Remove the hexane upper phase and take 50 µl of the lower phase for analysis.
  Note: Sample dilution factor: 2, minimum detection dose: 0.4 ppb.

- **Milk:** Dilute the milk 20-fold with 1× Re-dissolve solution (Solution 3) (i.e. dilute 25 µl milk in 475 µl Solution 3). Mix for 1 min to ensure that the mixture is fully dissolved. Take 50 µl for detection and analysis.
  Note: Sample dilution factor: 20, minimum detection dose: 3 ppb

- **Milk Powder:** Weigh 0.5 ± 0.02 g milk powder and add to a 10 ml tube. Add 5 ml deionized water and mix to ensure that the mixture is fully dissolved. Take 100 µl and add to 400 µl of 1× Re-dissolve solution (Solution 3) and mix thoroughly for 1 min. Take 50 µl for detection and analysis.
  Note: Sample dilution factor: 50, minimum detection dose: 6 ppb

- **Egg:** Weigh 1 ± 0.02 g of homogenate and add to a 10 ml tube. Add 5 ml deionized water and mix to ensure that the mixture is fully dissolved. Take 100 µl and add to 400 µl of 1× Re-dissolve solution (Solution 3) and mix thoroughly for 1 min. Take 50 µl for detection and analysis.
  Note: Sample dilution factor: 30, minimum detection dose: 3 ppb.

- **Urine:** Take 1 ml of clear urine into a tube, and add 4 ml of 1× Re-dissolve solution (Solution 3) and mix thoroughly for 30 seconds. Take 50 µl for detection and analysis.
  Note: Sample dilution factor: 1, minimum detection dose: 0.05 ppb

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 sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used. Samples that contain NaN_3 cannot be detected as it interferes with HRP.

» Always use non-pyrogenic, endotoxin-free tubes for blood collection.

Sample dilution guideline:

Estimate the concentration of the target in the sample and select the correct dilution factor to make the diluted target concentration fall near the middle of the kit's range. Generally, for high concentration (10000 pg/ml - 100000 pg/ml), dilute 1:100, for medium concentration (1000 pg/ml - 10000 pg/ml), dilute 1:10 and for low concentration (15.625 pg/ml - 1000 pg/ml), dilute 1:2. Very low concentrations (≤ 15.625 pg/ml) do not need dilution. Dilute the sample with the provided Sample Diluent Buffer and mix thoroughly. Several trials may be necessary to determine the optimal dilution factor.

3. Wash buffer

Dilute the concentrated Wash buffer 20-fold (1/20) with distilled water (i.e. add 40 ml of concentrated wash buffer into 760 ml of distilled water).

B. Assay Procedure

Bring all reagents to room temperature prior to use.

1. Set standard, test sample and control (zero) 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 prepared standards solutions into the standard wells.
3. Add 50 µl of PBS into the control (zero) well.
4. Add 50 µl of appropriately diluted sample into test sample wells.
5. Immediately add 50 µl of HRP conjugate reagent into each well, and then add 50 µl of Antibody solution. Add the solutions at the bottom of each well without touching the side wall.
6. Cover the plate with the plate sealer. Tap the plate gently to ensure thorough mixing. Incubate at 25°C for 45 minutes.
7. Remove the cover, and wash the plate 5 times. Discard the solution without touching the side walls. Blot the plate on an absorbent material. Add 250 µl of wash buffer to each well and soak for at least 1 min. Discard the contents and clap the plate on absorbent filter papers or other 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 min.

8. Add 50 µl of substrate solution A and 50 µl of substrate solution B into each well. 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.
9. Add 50 µl of Stop solution into each well (including the blank well). There should be a colour change to yellow. Gently mix the plate to ensure thorough mixing.

10. 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.

This assay is competitive, therefore there is an inverse correlation between QNs 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 solution A and substrate solution 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 dump the residual solution back into the vial.