Fluoroquinolones ELISA Kit

Catalog No: abx364905

Size: 96T

Detection Limit: Muscle - 0.6 ng/ml; Honey -0.8 ng/ml; Milk, Eggs - 6 ng/ml; Milk powder - 12 ng/ml; Urine - 1 ng/ml.

Sensitivity: 0.2 ng/ml (ppb)

Cross-reactivity: Enrofloxacin: 100%; Norfloxacin: > 100%; Ciprofloxacin: > 100%; Lomefloxacin: > 100%; Flumequine: > 100%; Peflacine: > 100%; Danofloxacin: > 100%; Sarafloxacin: > 100%; Difloxacin: 84%; Enoxacin: 66%; Ofloxacin (racemate): 58%; Oxolinic acid: 28%; Levofloxacin: 10%; Marbofloxacin: 4%

Sample recovery rate: Muscle, Honey, Milk, Milk powder, Eggs, Urine - 85% ± 15%.

Storage: Store all components at 4°C in the dark.

Application: The quantitative detection of Fluoroquinolones in Muscle, Honey, Milk, Milk powder, Eggs, and Urine samples.

Principle of the Assay: This kit is based on competitive enzyme-linked immuno-sorbent assay technology. An antigen is pre-coated onto a 96-well plate. Standards, test samples, and biotin-conjugated reagent are added to the wells and incubated. The HRP-conjugated reagent is then added, and the whole plate is incubated. Unbound conjugates are removed using wash buffer at each stage. TMB substrate is used to quantify the HRP enzymatic reaction. After TMB substrate is added, only wells that contain sufficient Fluoroquinolones will produce a blue coloured product, which then changes to yellow after adding the acidic stop solution. The intensity of the yellow colour is proportional to the Fluoroquinolones amount bound on the plate. The Optical Density (OD) is measured spectrophotometrically at 450 nm in a microplate reader, from which the concentration of Fluoroquinolones can be calculated.

Kit Components

- Pre-coated 96-Well Microplate: 12 x 8
- Standard: 6 × 1 ml (0 ppb, 0.2 ppb, 0.6 ppb, 1.8 ppb, 5.4 ppb, 16.2 ppb)
- Wash Buffer: (20X) 40 ml
- Reconstitution Buffer: (5X) 50 ml
- Detection Reagent A: 5.5 ml
- Detection Reagent B 5.5 ml
- TMB Substrate A: 6 ml
- TMB Substrate B: 6 ml
- Stop Solution: 6 ml
- Plate Sealer: 3
- Hermetic Bag: 1

Materials Required But Not Provided

- 37°C incubator
- Multi and single channel pipettes and sterile pipette tips
- Squirt bottle or automated microplate washer
- 1.5 ml tubes
- Distilled or deionized water
- Absorbent filter papers
- 100 ml and 1 liter graduated cylinders
- Microplate reader (wavelength: 450 nm)
- ELISA Shaker
- Nitrogen evaporators or water bath (50°C)

Reagents Required But Not Provided

- Acetonitrile
- N-hexane
- Hydrochloric acid
- Dichloromethane

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Protocol

A. Reagent Preparation

Prepare reagents fresh for each assay according to the number of samples required.

0.15 M HCl Solution: Dilute 5 ml of HCl to a final volume of 400 ml with deionized water and mix fully.

Extraction Solution: Add 10 ml of 0.15 HCl Solution to 90 ml of Acetonitrile and mix fully.

Reconstitution Buffer (1X): Dilute the 5X Reconstitution Buffer 5-fold with deionized water and mix fully. The Reconstitution Buffer (1X) may be stored at 4°C for up to one month.

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). If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

B. Sample Preparation

Analyse immediately or store samples at 2-8°C (within 24 hrs). For long term storage, aliquot and store at -20°C or -80°C. Avoid multiple freeze-thaw cycles.

- **Muscle:** Remove any fat from the sample, homogenize with a homogenizer. Weigh 2 g of the homogenate, add 8 ml of Extraction Solution, and vortex for 5 minutes to mix fully. Centrifuge at 4000 rpm for 10 minutes at room temperature. Take 2 ml of the clear upper layer supernatant to a dry glass tube, and dry at 50°C using Nitrogen Evaporators or a water bath. Dissolve the residue with 1 ml of N-hexane and vortex for 2 minutes. Add 1 ml of Reconstitution Buffer (1X) and vortex for 30 seconds to mix fully. Centrifuge at 4000 rpm for 5 minutes at room temperature. Discard the upper layer supernatant (N-hexane), and take 50 µl of the bottom layer supernatant for detection.
  Sample dilution factor: 2
- **Honey:** Weigh 1 g of honey, add 6 ml of Extraction Solution, and vortex for 5 minutes to mix fully. Add 3 ml of Reconstitution Buffer (1X) and 11 ml of Dichloromethane, and vortex for 5 minutes. Centrifuge at 4000 rpm for 5 minutes at room temperature. Take 8 ml of the clear upper layer supernatant to a dry glass tube, and dry at 50°C using Nitrogen Evaporators or a water bath. Dissolve the residue with 1 ml of Reconstitution Buffer, add 1 ml of N-hexane and vortex for 30 seconds. Centrifuge at 4000 rpm for 5 minutes at room temperature. Discard the upper layer supernatant (N-hexane), and take 50 µl of the bottom layer supernatant for detection.
  Sample dilution factor: 2
- **Milk:** Dilute 25 µl of milk with 475 µl of Reconstitution Buffer (1X). Vortex for 1 minute to mix fully. Take 50 µl for detection. *Sample dilution factor: 20*
- **Milk Powder:** Dissolve 0.5 g of milk powder sample with 5 ml of deionized water and vortex to dissolve fully. Mix 100 µl of the sample with 400 µl of Reconstitution Buffer (1X) and vortex for 1 minute. Take 50 µl for detection.
  Sample dilution factor: 50
- **Egg:** Add 1 g of egg sample with 5 ml of deionized water and vortex to dissolve fully. Mix 100 µl of the sample with 400 µl of Reconstitution Buffer (1X) and vortex for 1 minute. Take 50 µl for detection.
  Sample dilution factor: 30
- **Urine:** If urine contains particulates, filter or centrifuge at 4000 rpm for 5 minutes and take the supernatant. Dilute 1 ml of clear urine with 4 ml of Reconstitution Buffer (1X). Vortex for 30 seconds to mix fully. Take 50 µl for detection.
  Sample dilution factor: 5

Notes:

- Samples must be diluted so that the expected concentration falls within the kit's range.
- Always use non-pyrogenic, endotoxin-free tubes for blood collection.
- Fresh samples, or recently obtained samples, are recommended to prevent protein degradation and denaturation that may lead to erroneous results.
- NaN_3 cannot be used as a test sample preservative, since it inhibits HRP.
- If possible, prepare solid samples using sonication and/or homogenization, as lysis buffers may (on occasion) interfere with the kit's performance.
- If a sample is not indicated in the manuals applications, a preliminary experiment to determine the suitability of the kit will be required.

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C. Assay Protocol

Prepare all standards, samples and reagents as directed above. Equilibrate the kit components and samples to room temperature prior to use. It is recommended to measure in duplicate, and to plot a standard curve for each test.

1. Set standard and test sample wells on the pre-coated plate respectively, and record their positions. *Add the solution 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 standards into the standard wells.
3. Aliquot 50 µl of appropriately prepared sample into the test sample wells.
4. Aliquot 50 µl of Detection Reagent B to each well, then aliquot 50 µl of Detection Reagent A to each well.
5. Gently tap the plate to mix, or use a microplate shaker. Cover the plate with a plate sealer and incubate for 45 mins at 25°C in the dark.
6. Remove the cover and discard the solution. Wash the plate 5 times with 1X Wash Buffer. *Fill each well completely with Wash buffer (300 µl) using a multi-channel Pipette or autowasher (30 secs 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 50 µl of TMB Substrate A to each well, then aliquot 50 µl of TMB Substrate B to each well. Seal the plate and incubate at 25°C in the dark for 10-20 minutes. The incubation time is for reference only, the optimal time should be determined by end user. Avoid exposure to light.
8. 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.
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 OD at 450 nm immediately. Measure the OD within 10 minutes of adding the Stop Solution.

For calculation, average the OD 450 readings for each reference standard, and each sample, and then subtract the average control (zero) OD reading.

(Relative OD) = (OD of Each Well) – (OD of Zero Well)

The standard curve can be plotted as the relative OD of each reference standard solution (X), against the respective concentration of each standard solution (Y). The concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the concentrations from interpolation by the dilution factor, to obtain the concentration before dilution.

Precautions:

- Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
- Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not exceed 10 mins.
- Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled.
- Do not reuse pipette tips and tubes.
- Do not use expired components, or components from a different kit.
- The TMB substrate should be used under sterile conditions, and light exposure should be minimised. Unused substrate should be colorless, or a very light yellow in appearance. Do not discard any residual solution back into the vial.
- Please note that this kit is optimised for detection of native samples, rather than recombinant proteins or synthetic chemicals. We are unable to guarantee detection of recombinant proteins, as they may have different sequences or tertiary structures to the native protein.

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Precision:

Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of Fluoroquinolones were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of Fluoroquinolones were tested on 3 different plates, 8 replicates in each plate.

CV (%) = (Standard Deviation / Mean) × 100

Intra-Assay: CV < 10%

Inter-Assay: CV < 10%

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