Intended Use

The Beacon Fluoroquinolones (FQS) Plate Kit is an immunoassay for the detection of FQS in food samples. This product is intended for research use only.

Principles

FQS HRP Enzyme Conjugate is pipetted into the test wells followed by the Calibrators and the Sample Extract(s). A soluble polyclonal FQS antibody solution is then added to the test wells to initiate the reaction. During an incubation, FQS and FQS HRP Enzyme Conjugate compete for binding to the soluble FQS antibody which is in turn immobilized on the test wells. Following the incubation, the wells are washed to remove any unbound FQS and FQS HRP Enzyme Conjugate. After washing, a colorless substrate is added to the wells and any bound enzyme conjugate will convert the substrate to a blue color. Following an incubation, the reaction is stopped with the addition of Stop Solution and the amount of color in each well is measured. The color of the unknown sample is compared to the color of the calibrators and the FQS concentration of the sample is derived.

Reagents and Materials Provided

1 Unit	Plate containing 12 test strips of 8 wells each that are vacuum sealed in an aluminized pouch with a desiccant.
6 X 2 mL	Vials of FQS Calibrators (0, 0.2, 0.8, 3.2, 6.4, 16 ppb).
1 X 8 mL	Bottle of FQS HRP Enzyme Conjugate.
1 X 8 mL	Bottle of FQS Antibody.
1 X 14 mL	Bottle of Substrate.
1 X 14 mL	Bottle of Stop Solution.

Reagents and Materials Required but Not Provided

- Pipette(s) with disposable tips capable of dispensing the required volume(s).
- Multichannel pipette(s) (8 channels) with disposable tips capable of dispensing the required volume(s) (recommended if running more than two strips at once).
- Laboratory quality distilled or deionized water.
- Reagents and materials for sample preparation.
- Personal protective equipment.
- Paper towels or equivalent absorbent material.
- Wash bottle (optional).
- Timer.
- Microtiter plate or strip reader capable of reading at 450 nm.

Kit Handling Notes and Precautions

- Read the product brochure in its entirety prior to use.
- The kit, in its original packaging, can be used until the end of the month indicated on the box label.
- Do not use reagents after expiration date.
- Store all kit components at 2°C to 8°C (36°F to 46°F) when not in use.
- Reagents should be brought to room temperature, 20°C to 28°C (68°F to 82°F), prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- Running Calibrators and Samples in duplicate will improve assay precision and accuracy.
- Precise transfer of samples and reagents by using a calibrated pipette that is capable of dispensing the required volume is critical to obtain proper assay results.
- If running more than two strips at once, the use of a multi-channel pipette is recommended when adding the Antibody, Substrate and Stop Solution.
- All procedural steps should be completed without interruption. Ensure all reagents, materials and equipment are ready at the appropriate time.
- Each reagent is optimized for use in the Beacon FQS Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon FQS Plate Kits with different lot numbers.
- Do not reuse test wells.
- Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- Damage to or obstruction of the optical surface may cause unsatisfactory results.

Performance Characteristics

The Beacon FQS Plate Kit cannot differentiate between the various Fluoroquinolones but detects their presence to differing degrees. The following table shows the relative values for the percent cross reactivity versus Ciprofloxacin.

Compound	% Cross-Reactivity
Ciprofloxacin	100
Enrofloxacin	123
Ofloxacin	77
Norfloxacin	168
Danofloxacin	105
Lomefloxacin	96
Enoxacin	96
Oxolenic Acid	37
Difloxacin	37
Levofloxacin	20
Flumequine	9

Limit of Detection (LOD)

Matrix	LOD (ppb)
Meat	1
Fish	1
Shrimp	1
Honey	2

Sample Buffer Preparation (10 mM PBS)

1. Measure 1 L of laboratory quality distilled or deionized water and add to a clean container with a tight fitting lid.
2. Weigh 0.34 g of $NaH_2PO_4 \cdot H_2O$ (F.W. 137.99) and add to the container.
3. Weigh 1.08 g of Na_2HPO_4 (F.W. 141.96) and add to the container.
4. Weigh 8.5 g of NaCl and add to the container.
5. Gently stir to mix.
6. Measure the pH of the solution and adjust to achieve a pH of 7, as necessary.

Sample Preparation

Meat: (Dilution Factor: 2)

1. Homogenize the sample.
2. Weigh 5 g of the homogenized sample into a 50 mL conical tube.
3. Measure 10 mL of Acetonitrile and add to the conical tube.
4. Mix vigorously for 5 minutes.
5. Centrifuge for 5 minutes at 3,000 X g.
6. Transfer 2 mL of the clear supernatant to a clean conical tube.
7. Treat the sample with nitrogen steam until dry.
8. Measure 2 mL of hexane and add to the conical tube. Mix vigorously for 1 minute.
9. Measure 2 mL of Sample Buffer and add to the conical tube. Mix vigorously.
10. Centrifuge for 5 minutes at 3,000 X g.
11. Discard the upper layer and transfer the lower phase to a clean vial for use in the assay.

Honey: (Dilution Factor: 2)

1. Weigh 1 g of sample into a 50 mL conical tube.
2. Measure 2 mL of Sample Buffer and add to the conical tube. Mix vigorously.
3. Measure 8 mL of dichloromethane and add to the conical tube. Mix vigorously.
4. Centrifuge for 5 minutes at 3,000 X g.
5. Discard the upper layer and transfer the lower phase to a clean vial.
6. Treat the sample with nitrogen steam until dry.
7. Measure 2 mL of Sample Buffer and add it to the vial. Mix vigorously and use in the assay.

Assay Procedure

1. Allow kit components and the sample extract(s) to reach room temperature prior to running the test.
2. Place the appropriate number of test wells into a holder. Be sure to re-seal unused test wells in the zip-lock bag with the desiccant to limit exposure to moisture.
3. Dispense **50 µL of Enzyme Conjugate** into each well.
4. Dispense **50 µL of Calibrators and Sample Extract(s)** into the appropriate well. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
5. Dispense **50 µL of Antibody** into each well.
6. Gently shake the wells for 30 seconds using a back-and-forth motion and incubate for **30 minutes** at room temperature.
7. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with laboratory quality distilled or deionized water and then decant. Repeat this wash step four times for a total of five washes. Following the last wash, tap the inverted wells onto absorbent paper to remove excess wash solution.
8. Dispense **100 µL of Substrate** into each well.
9. Incubate for **30 minutes** at room temperature.
10. Dispense **100 µL of Stop Solution** into each well in the same order of addition as the Substrate.
11. Gently shake the wells for 30 seconds using a back-and-forth motion.
12. Carefully wipe the optical surface with a soft, lint-free wipe. Measure and record the absorbance (Optical Density; OD) of each well at 450 nm using a plate or strip reader within 10 minutes of stopping the assay. If the reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm.
13. Dispose of used test wells in an appropriate waste container.

Quality Control (QC) Criteria

- The correlation coefficient (R^2) of the calibration curve, analyzed using a 4-parameter logistic regression, must be ≥ 0.99.
- The average absorbance of the zero calibrator replicates must be ≥ 1.0.
- The average absorbance of calibrator replicates must have a coefficient of variation (%CV) < 15%.
- The average absorbance of sample replicates must have a coefficient of variation (%CV) < 20%.

Result Interpretation

It is preferred for quantitative results to be determined using commercially available software for ELISA evaluation using a 4-parameter curve fit. Alternatively, a semi-log curve fit can be used if 4-parameter software is not available. A spreadsheet that will perform the curve fit and sample concentration calculations is available upon request. Please contact Beacon for further details.

To ensure the validity of the results, please adhere to the following:

- Ensure QC criteria are met.
- The concentration of FQS in a sample is determined by comparing the average sample absorbance to the standard curve. This value must then be multiplied by the dilution factor used.
- In the event that the average absorbance of the sample is lower than the highest calibrator, further dilute the sample extract to fit into the standard curve and retest alongside the calibrators. Sample results must be multiplied by the total dilution factor used.

Technical Assistance
For questions regarding this kit or for additional information about Beacon products, contact us.

Beacon Analytical Systems, Inc.
82 Industrial Park Rd. Saco, ME 04046
Tel. +1-207-571-4302
info@beaconkits.com | www.beaconkits.com

Safety
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