

## RESULTS INTERPRETATION

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator wells: Sample containing less color than a calibrator well have a concentration of Alachlor greater than the concentration of the calibrator. Samples containing more color than a calibrator well have a concentration less than the concentration of the calibrator.
2. Quantitative interpretation requires graphing the absorbances of the calibrators (y axis) versus the log of the calibrator concentration (x axis) on semi-log graph paper. A straight line is drawn through the calibrator points and the sample absorbances are located on the line. The corresponding point on the x axis is the concentration of the sample. Samples with absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as < 0.2ppb or >16 ppb, respectively.

## SAMPLE CALCULATIONS

Well Contents	OD <sub>450</sub>	Average OD ± SD*	%RSD	%Bo**
0 ppb Calibrator	1.341 1.270	1.305 ± 0.050	3.8	100
0.2 ppb Calibrator	1.085 1.096	1.090 ± 0.008	0.7	83.5
0.8 ppb Calibrator	0.911 0.868	0.890 ± 0.030	3.4	68.1
3.2 ppb Calibrator	0.607 0.602	0.604 ± 0.004	0.6	46.3
6.4 ppb Calibrator	0.464 0.468	0.466 ± 0.003	0.6	35.7
16 ppb Calibrator	0.297 0.306	0.301 ± 0.006	2.1	23.1

This data is for example purposes only.

\* %Bo = (OD<sub>450</sub> / 0 ppb OD<sub>450</sub>)\*100

## TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Beacon products, call (207) 571-4302.

### SAFETY

To receive complete safety information on this product, contact Beacon Analytical Systems, Inc. and request Material Safety Data Sheets. Stop Solution is 1N hydrochloric acid. Handle with care.

### General Limited Warranty

Beacon Analytical Systems, Inc. ("Beacon") warrants the products manufactured by it against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product's printed expiration date. BEACON MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of Beacon products appearing in published catalogues and product literature may not be altered except by express written agreement signed by an officer of Beacon. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and, if given, should not be relied upon.

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## Fluoroquinolones Plate Kit Cat.# 20-0152

Instructional Booklet

READ COMPLETELY BEFORE USE.

### INTENDED USE

The Beacon Fluoroquinolones Plate Kit is a competitive ELISA for the quantitative analysis of Fluoroquinolones in food products.

## ASSAY PRINCIPLES

The Beacon Fluoroquinolones plate kit is a competitive enzyme-labeled immunoassay. Fluoroquinolones is extracted from a sample by blending or shaking with extraction solution. Fluoroquinolones-HRP enzyme conjugate is pipetted into the test wells followed by calibrators or sample extracts. Fluoroquinolones antibody is then pipetted into the test wells to initiate the reaction. During the 30 minute incubation period, Fluoroquinolones from the sample and Fluoroquinolones-HRP enzyme conjugate compete for binding to Fluoroquinolones antibody which, in turn, binds to the test well. Following this 30 minute incubation, the contents of the well are removed and the wells are washed to remove any unbound Fluoroquinolones and Fluoroquinolones HRP conjugate. A clear substrate is then added to the wells and any bound enzyme conjugate causes the conversion to a blue color. Following a 30 minute incubation, the reaction is stopped and amount of color in each well is read. The color of unknown samples is compared to the color of the calibrators and the Fluoroquinolones concentration of the samples is derived.

## SPECIFICITY

The Beacon Fluoroquinolones Plate Kit cannot differentiate between the various Fluoroquinolones, but detects their presence to differing degrees. The following table shows the relative values for the % cross reactivity (% CR) versus Ciprofloxacin. All concentrations are in parts per billion (ppb).

Compound	% CR
Ciprofloxacin	100
Enrofloxacin	123
Ofloxacin	77
Norfloxacin	168
Danofloxacin	105
Lomefloxacin	96
Enoxacin	96
Oxolenic acid	37
Difloxacin	37
Levofloxacin	20
Flumequine	9

## DETECTION LIMIT:

**Meat, Fish and Shrimp: 1 ppb**

**Honey: 2 ppb**

## PRECAUTIONS

1. Each reagent is optimized for use in the Beacon Fluoroquinolones Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Fluoroquinolones Plate Kits with different lot numbers.
2. Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
3. Do not use reagents after expiration date.
4. Reagents should be brought to room temperature, 20 – 28°C (62 – 82°F) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.

## REAGENTS AND MATERIALS PROVIDED

The kit in its original packaging can be used until the end of the month indicated on the box label when stored at 2 – 8°C.

- 1 plate containing 12 test strips of 8 wells each vacuum-packed in an aluminized pouch with indicating desiccant
- 1 vial of Negative control (0.0 ppb ciprofloxacin)
- 1 vial each of calibrators 0.2 ppb, 0.8 ppb, 3.2 ppb, 6.4 ppb and 16 ppb ciprofloxacin
- 1 vial of Fluoroquinolones-HRP enzyme conjugate
- 1 vial of Rabbit anti-Fluoroquinolones antibody
- 1 vial of Substrate
- 1 vial of Stop Solution (Caution! 1N HCl. Handle with care.)
- 1 Instructional booklet

## MATERIALS REQUIRED BUT NOT PROVIDED

- Laboratory quality distilled or deionized water.
- Graduated cylinder, 100 ml or larger.
- Glassware for sample extraction and extract collection.
- Centrifuge.
- Pipet with disposable tips capable of dispensing 50 µL.
- Multi-channel pipet; 8 channel capable of dispensing 50 and 100 µL.
- Paper towels or equivalent absorbent material.
- Microwell plate or strip reader with 450nm filter.
- Timer
- Blender
- Wash Bottle
- Acetonitrile
- Hexane
- Dichloromethane
- Distilled water
- 10 mM PBS, [0.31 g NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O+ 2.87 g Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O + 9 g NaCl, filling to 1 liter with distilled water]

## SAMPLE PREPARATION

### Meat sample (1:2 dilution)

1. Homogenize the sample.
2. Weigh 5 g homogenized sample into a 50 mL centrifugal tube and add 10 mL of Acetonitrile.
3. Mix vigorously for 5 minutes.
4. Centrifuge 5 min at 3000 g at room temperature.
5. Transfer 2 mL of the clear supernatant to dry with gentle nitrogen stream until dryness.
6. Add 2 mL of Hexane to the tube and mix vigorously for 1 min and add 2 mL 10 mM PBS. Mix vigorously.
7. Centrifuge for 5 min at 3000 g at room temperature.
8. Discard the upper layer.
9. Transfer the lower phase into a clean vial and pipette 50 µL per well for the assay.

### Honey sample (1:2 dilution)

1. Weigh 1 gram of honey in a screw cap glass bottle (15 mL size).
2. Add 2 mL of 10 mM PBS and mix vigorously.
3. Add 8 mL of dichloromethane and mix vigorously.
4. Centrifuge for 5 minutes at 3000 g at room temperature.
5. Discard the upper layer and transfer the lower phase to a clean vial. Dry using a gentle nitrogen stream until completely dry.
6. Redissolve the dry extract with 2 ml of 10 mM PBS. Mix vigorously.
7. Pipette 50 µL per well for the assay.

## ASSAY PROCEDURE

(Note: Running calibrators and samples in duplicate will improve assay precision and accuracy.)

1. Allow reagents and sample extracts to reach room temperature prior to running the test.
2. Place the appropriate number of test wells into a microwell holder. Be sure to re-seal unused wells in the zip-lock bag with dessicant.
3. Dispense **50 µL of Enzyme Conjugate** into each test well.
4. Using a pipet with disposable tips, add **50 µL of calibrators and samples** to the appropriate test wells. Be sure to use a clean pipet tip for each.
5. Dispense **50 µL of Antibody Solution** into each test well.
6. Incubate the test wells for **30 minutes**.
7. Dump the contents of the wells into an appropriate waste container. Fill the wells to overflowing with distilled water and dump. Repeat 3X for a total of four washes.
8. Following the last wash tap the inverted wells onto absorbent paper to remove the last of the wash solution.
9. Dispense **100 µL of Substrate** into each well.
10. Incubate the wells for **30 minutes**.
11. Dispense **100 µL of Stop Solution** into each test well.
12. Read and record the absorbance of the wells at 450nm using a strip or plate reader.