Quinolones ELISA Kit

Enzyme Immunoassay for the quantification determination of Pecan Nut in food

Catalog number: ARG83052

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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SECTION

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INTRODUCTION

Quinolones, highly effective synthetic antibiotics, are one of the most commonly prescribed antibiotic classes in outpatient and in acute care

hospital settings. They inhibit DNA gyrase in Gram-negative bacteria and topoisomerase IV in Gram-positive bacteria, promoting the DNA cleavage and rapid killing of susceptible bacteria. With their broad-spectrum activity against both Gram-positive and Gram-negative aerobic and anaerobic bacteria, excellent tissue and intracellular penetration, high bioavailability, and generally good oral tolerability, quinolones have a broad array of indications including treatment of urinary tract infections (UTIs), sexually transmitted diseases, gastrointestinal and abdominal infections, respiratory tract infections, bone and joint infections, and skin and soft tissue infections in adults. Although considered well-tolerated in general, quinolones can induce allergic reactions. In fact, they are the second most common antibiotics associated with allergic reactions, following beta-lactams. Currently, most of the data regarding antibiotic allergy have been published for patients with a beta-lactam allergy and the paucity of data exist regarding quinolone allergy.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative competitive enzyme immunoassay technique. Quinolones antibody is bind on the surface of a microtiter plate. Quinolones containing samples or standards, Quinolones -Peroxidase and an antibody directed against Quinolones are given into the wells of the microtiter plate. Immobilized and free Quinolones compete for the antibody binding sites. After 30 minutes incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. Then a substrate solution is added and incubated for 15 minutes, resulting in the evelopment of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured at 450 nm.

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The concentration of Quinolones is indirectly proportional to the color intensity of the test sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard A-F (0, 0.4, 1, 4, 10, 40 ng/ml)	6 X 1 ml (Ready-to-use)	4°C.
5X Sample Diluent Buffer	120 ml (Ready-to-use)	4°C
HRP-Quinolones Conjugate	15 ml (Ready-to-use)	4°C
10X Wash buffer	60 ml	4°C
TMB substrate	15 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	15 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C in the dark at all times. Do not expose reagents to heat, sun, or strong light during storage and usage.

- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.
- Briefly spin down the antibody conjugate Mixture and HRP-Streptavidin concentrate before use.
- All materials must be at room temperature (20-28°C) prior to use.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- All incubation steps must be accurately timed.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

Due to high risk of cross-contamination all applied instruments like applicator, mortar, glass vials etc. have to be cleaned thoroughly before and after each sample. Quinolones adhere very strongly to different surfaces. In certain cases they can resist a common dishwasher cleaning. To identify possible crosscontamination caused by previous extractions it is strongly recommended to note the sequence of the extractions. The following sample preparation should be applied for all kinds of samples:

Honey - Add honey into 70% methanol and mix 5 minutes at room temperature. After that, centrifuge at of 3000 g for 15 minutes. Collect the supernatant and diluted with appropriate dilution fold.

Milk / Serum / Other liquid samples - Defat milk is applicable. If sample is not Defat milk, the milk need be centrifuged for at 2000 g, 4°C for 15 min. Afterwards the upper fat layer should be removed. Dilute with 1X Sample

Diluent Buffer and mix with suspension for 5 minutes at room temperature. Then the sample need centrifuge at 3000 g for 15 minutes. For a better separation of fat the centrifuge should be cooled to 4°C if applicable. Apply the filtrate supernatant in the ELISA.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 10 ml of 10X Wash buffer + 90 ml of distilled water) The diluted Wash buffer is stable for 30 days at 2°C to 8°C.
- 1X Sample Diluent buffer: Dilute 5X Sample Diluent concentrate into distilled water to yield 1X Sample Diluent buffer. (E.g. 10 ml of 5X Sample Diluent concentrate + 40 ml of distilled water) The Sample diluent buffer is stable for 7 days at 2°C to 8°C.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-28 °C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 50 µl of standards or prepared samples into wells.
3. Add 100 µl of HRP-Quinolones Conjugate into wells.
4. Incubate for 30 minutes at RT.
5. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (300µl) using a

squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

6. Add 100 µl of TMB Reagent to each well.
7. Incubate for 15-20 minutes at RT in dark.
8. Add 100 µl of Stop Solution to each well. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at 450 nm immediately (optional: Read the OD at 600-690nm as reference). It is recommended read the absorbance within 30 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

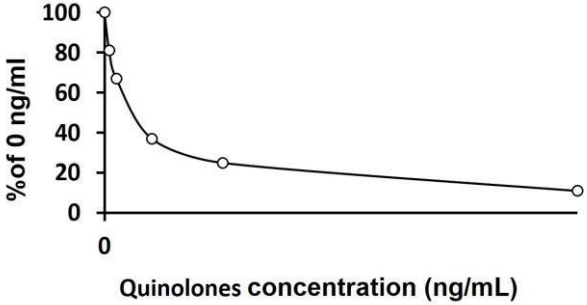
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details.

(https://www.arigobio.com/elisa-analysis)

6. If these quality control criteria are not met the assay run is invalid and should be repeated.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The standard range of Quinolones ranged from 0.4 -40ng/ml. The mean Limit of detection was 0.13ng/ml.

Linearity

71% - 92%.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 4.3% and inter-assay precision was 11.2%.