Version 20-04/24

User's Manual

Ciprofloxacin ELISA Kit

REF **DEIANJ04**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This ELISA Kit is for the quantitative detection of Ciprofloxacin (CPFX) concentration in tissues, honey, milk, milk powder, egg.

General Description

Ciprofloxacin is an antibiotic used to treat a number of bacterial infections. This includes bone and joint infections, intra abdominal infections, certain type of infectious diarrhea, respiratory tract infections, skin infections, typhoid fever, and urinary tract infections, among others. For some infections it is used in addition to other antibiotics. It can be taken by mouth or used intravenously.

Principles of Testing

This assay is based on the competitive enzyme immunoassay for the detection of Ciprofloxacin in the sample. The coupling antigens are pre-coated on the micro-well stripes. The Ciprofloxacin in the sample and the coupling antigens pre-coated on the micro-well stripes compete for the anti-Ciprofloxacin antibodies. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the sample has a negative correlation with the Ciprofloxacin in it. This value is compared to the standard curve and the Ciprofloxacin concentration is subsequently obtained.

Reagents And Materials Provided

Assay plate (96 Wells) 1

Instruction manual 1

Standard 6 x 1 mL 8.1 ppb, 2.7 ppb, 0.9 ppb, 0.3 ppb, 0.1 ppb, 0 ppb

Redissolving Solution (concentrate 5 x) 2 x 20 mL

Antibody 1 x 6 mL

HRP-Conjugate 1 x 6 mL

Wash Buffer (concentrate 20 x) 1 x 20 mL

Stop Solution 1 x 6 mL

Substrate A 1 x 6 mL

Substrate B 1 x 6 mL

Adhesive Films 4

Materials Required But Not Supplied

Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at the dual-wavelength 450/630 nm

Equipments: microplate reader, printer, homogenizer, nitrogen-drying device, vortex, centrifuge, measuring pipets, balance (a reciprocal sensibility of 0.01 g)

Micropipettors: single-channel 20-200 µL, 100-1000 µL, and multi-channel 250 µL

Reagents: dichloromethane, N-hexane, acetonitrile 99.9%, HCl (approx 36.5%).

Storage

Store at 2 - 8°C. Do not use past kit expiration date.

Specimen Collection And Preparation

Instructions (The following points must be dealt with before the pre-treatment of any kind of sample):

1. Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents;
2. Before the experiment, each experimental utensil must be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.

Solution preparation before sample pre-treatment:

1. 0.15 M HCl solution: dissolve 5 mL HCl (36.5%) in deionized water to 400 mL.
2. Sample extract: dissolve 10 mL 0.15 M HCl solution in acetonitrile to 100 mL, mix.
3. Redissolving solution (1 x): dilute the 5× concentrated redissolving solution with deionized water at 1:4 (1 mL 5×concentrated redissolving solution+4 mL deionized water), used for sample redissolving.
4. Washing buffer: dilute 20 mL of the concentrated washing buffer (20×concentrated) with the distilled or deionized water at 1:19 to 400 mL (or just to the required volume) for use.

Samples preparation:

Tissue (chicken, shrimp, fish, liver, etc) :

1. Homogenize the samples.
2. Weigh 2.0 ± 0.05 g of the homogenized samples into 50 mL centrifugal tube.
3. Add 8 mL of the Sample extract, shake properly for 5 minutes, centrifuge at above 4000 r/min at room temperature (20-25°C) for 10 minutes.
4. Take 2 mL of the supernatant, into a new centrifugal tube, and evaporate to dryness by nitrogen or air at 56°C.
5. Dissolve the dry residues in 1 mL N-hexane, shake properly for 2 minutes, add 1 mL of the diluted redissolving solution, mix properly for 30s, centrifuge at above 4000 r/min at room temperature (20-25°C) for 5 minutes.
6. Discard the whole upper layers, take 50 µL of the lower layer for further analysis.

Fold of dilution of the sample: 2

Honey:

1. Take 1 ± 0.05g honey into 50 mL centrifugal tube, add 6 mL Sample extract, shake properly for 5 minutes, dissolve it completely.
2. Add 3 mL of the diluted redissolving solution, add 11 mL dichloromethane, vortex for 5 minutes and

centrifuge at above 4000 r/min at room temperature (20-25°C) for 5 minutes.

3. Remove the upper layer, and take 8 mL of the organic phase (lower layer) into a new centrifugal tube, and evaporate to dryness by nitrogen or air at 56°C.
4. Dissolve the dry residues in 1 mL of the diluted redissolving solution, shake properly for 2 minutes, add 1 mL N-hexane, mix properly for 30s, centrifuge at above 3000 r/min at room temperature (20-25°C) for 5 minutes.
5. Discard the whole upper layers, take 50 µL of the lower layer for further analysis.

Fold of dilution of the sample: 2

Milk;

1. Take 5 mL sample into 50 mL centrifugal tube, centrifuge at above 4000 r/min at 15°C for 10 minutes, remove fat (upper layer).
2. Transfer 25 µL milk removed fat into 2 mL centrifuge tube. Add 475 µL of the diluted redissolving solution, shake strongly for 1 minute, dissolve it completely.
3. Take 50 µL for further analysis.

Fold of dilution of the sample: 20

Milk powder;

1. Weigh 0.5 ± 0.02 g milk powder into 10 mL centrifuge tube, add 5 mL of the deionized water, shake strongly for 1 minute, dissolve it completely.
2. Transfer 100 µL of the sample solution into 2 mL centrifuge tube. Add 400 µL of the diluted redissolving solution, shake strongly for 1 minute.
3. Take 50 µL for further analysis.

Fold of dilution of the sample: 50

Egg;

1. Homogenize the sample (Egg white, egg yolk or whole egg).
2. Weigh 1.0 ± 0.02 g of the homogenized sample into 10 mL centrifugal tube, add 5 mL of the deionized water, shake strongly for 1 minute, dissolve it completely.
3. Transfer 100 µL of the sample solution into 2 mL centrifuge tube. Add 400 µL of the diluted redissolving solution, shake strongly for 1 minute.
4. Take 50 µL for further analysis.

Fold of dilution of the sample: 30

【SAMPLE STORAGE】

1. Untreated samples are stored at frozen environment.
2. Prepared sample can be stable at 2-8°C for 1 week.

Assay Procedure

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

1. Bring test kit to the room temperature (20-25°C) for at least 30 minutes, note that each reagent must be

shaken evenly before use; put the required micro-well strips into plate frames. Re-sealed the unused microplate, stored at 2-8°C, not frozen.

2. Add 50 µL of the sample or standard solution into separate wells; add 50 µL of HRP-Conjugate, then add 50 µL of the antibody working solution into each well, mix gently by shaking the plate manully. Seal the microplate with the adhesive films, and incubate at 25°C for 45 minutes.
3. Pour liquid out of microwell, add 250 µL/well of washing buffer to wash microplate for 15-30s, repeat 4-5 times, flap to dry with absorbent paper at last.
4. Add 50 µL of Substrate A and 50 µL of Substrate B to each well, mix well. Incubate for 15 minutes at 25°C. Keeping the plate away from drafts and other temperature fluctuations in the dark. Avoid placing the plate in direct light.
5. Add 50 µL of Stop Solution to each well. when the first four wells containing the highest concentration of standards develop obvious blue color. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
6. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 630 nm. Subtract readings at 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Calculation

There are two methods to judge the results; the first one is the rough judgment, while the second is the quantitative determination. Note that the OD value of the sample has a negative correlation with the Ciprofloxacin concentration in the sample.

1) Qualitative determination

The concentration range (ppb) of Ciprofloxacin can be obtained from comparing the average OD value of the sample with that of the standard solution.

2) Quantitative determination

The mean values of the absorbance values obtained for the average OD value (B) of the sample and the standard solution divided by the OD value (B0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is

Percentage of absorbance value = B/B0 ×100%

B—the average (double wells) OD value of the sample or the standard solution

B0—the average OD value of the 0 ppb standard solution

Draw the standard curve with the absorption percentages of standard solutions and the semilogarithm values of Ciprofloxacin standard solutions (ppb) as Y- and X-axis, respectively. Read the corresponding concentration of the sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the dilution fold, finally obtaining Ciprofloxacin concentration in the sample.

Using the professional software of this kit will be more convenient for accurate and rapid analysis of a large amount of samples.

Precision

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Detection Limit

Tissue 0.3 ppb

Honey 0.4 ppb

Milk 3 ppb

Milk powder 6 ppb

Egg 3 ppb

Sensitivity

0.1ppb

Specificity

CPFX 100%

Oxolinic Acid 28%

Levofloxacin 10%

Lomefloxacin 4%

Marbofloxacin 4%

Sarafloxacin 2%

Limitations

FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

- The kit should not be used beyond the expiration date on the kit label.

- Do not mix or substitute reagents with those from other lots or sources.

- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.

- If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.

- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the ELISA Kit, the possibility of interference cannot be excluded.