ab285266 – Ciprofloxacin ELISA Kit

For the quantitative measurement of Ciprofloxacin in tissues, honey and urine.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285266

Storage and Stability

The entire kit may be stored at 4°C for up to 12 months from the date of shipment.

Materials Supplied

Item	Quantity	Storage Condition
Micro ELISA Plate	8 ×12 strips	4°C
Standard (S1 – S6)	6 x 1 mL	4°C
High Standard (100 ppb)	1 mL	4°C
Antibody Working Solution	5.5 mL	4°C
Enzyme Conjugate	5.5 mL	4°C
Substrate A Solution	6 mL	4°C
Substrate B Solution	6 mL	4°C
Stop Solution	5 mL	4°C
20X Concentrated Wash Solution	40 mL	4°C
5X Concentrated Redissolving Solution	50 mL	4°C
Plate Sealer	1 unit	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Reagents: 0.15M HCl, anhydrous acetonitrile, N-hexane, dichloromethane (CH_2Cl_2)
- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes with disposable tips
- Clean Eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

Reagent Preparation

- Prepare reagents within 30 minutes before the experiment.
- Before using the kit, spin the tubes prior to opening.

Standards: Standards are ready to use:

Tube #	S1	S2	S3	S4	S5	S6
Concentration (ng/mL)	0	0.1	0.3	0.9	2.7	8.1

Redissolving Solution: Dilute the concentrated redissolving solution 5 times with deionized water to be used for sample redissolving, it can be stored at 4°C environment up to a month.

Wash Buffer: Dilute 40 mL of the concentrated washing buffer with the distilled or deionized water to 800 mL (or just to the required volume) for using.

Sample Extracting Solution: Mix 10 mL 0.15M HCl with 90 mL anhydrous acetonitrile, mix completely.

Sample Preparation

- Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
- Avoid multiple freeze-thaw cycles.

Tissue, liver, egg samples: Weigh 2 g homogeneous sample into 50 mL centrifuge tube. Add 8 mL sample extract solution, mix with vortex for 5 minutes, centrifuge at 4000 r/minutes at room temperature for 10 minutes. Take 2 mL clear organic phase of upper into a 10 mL glass tube, and dry at 50 to 60°C with nitrogen or water bath. Add 1 mL N-hexane, mix with vortex for 2 minutes, then add 1 mL redissolving solution, mix with vortex for 30 seconds, centrifuge at 4000 r/minutes at room temperature for 5 minutes. Wipe out the upper N-hexane; take 50 µl Lower water phase for analysis (Dilution factor: 2).

Honey: Weigh 1.0 g homogeneous sample into 50mL centrifuge tube, add 6 mL sample extract solution, oscillate 5 minutes to make it dissolve completely. Add 3 mL redissolving solution, then add 11 mL dichloromethane (CH_2CL_2), oscillate 5 minutes, centrifuge at 4000 r/minutes at room temperature for 5 minutes. Wipe out the upper phase, take 8 mL organic phase to dry container, and dry at 50 to 60°C with nitrogen or water bath. Dissolve the dry residue with 1 mL redissolving solution, then add 1 mL N-hexane, mix 30s, centrifuge at 3000 r/minutes at room temperature for 5 minutes. Wipe out the upper phase; take 50 µl Lower phase for analysis (Dilution factor: 2).

Milk Sample: Take 25 µl milk sample and 475 µl redissolving solution, mix and oscillate for 1 minute. Use 50 µl solution for analysis (Dilution factor: 20).

Milk Powder: Weigh 0.5 g homogeneous sample into 10 mL centrifuge tube, add 5 mL deionized water and oscillate. Take 100 µl sample and 400 µl redissolving solution (Liquor 3), mix and oscillate for 1 minute. Use 50 µl solution for analysis (Dilution factor: 50).

Egg sample: Weigh 1.0 g homogeneous sample into 10mL centrifuge tube, add 5 mL deionized water, oscillate and dissolve fully. Take 100 µl sample and 400 µl redissolving solution, mix and oscillate for 1 min. Use 50 µl solution for analysis (Dilution factor: 30).

Assay Protocol

- Bring all reagents and samples to room temperature 30 minutes prior to the assay.
- Shake the reagent bottles to dissolve any crystals.
- It is recommended that all standards and samples be run at least in duplicate.
- A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Add 50 µL diluted standards or samples into marked well. Add 50 µL Enzyme conjugate and 50 µL antibody working solution into each well.
3. Oscillate the plate for 5 sec, cover the well and incubate in dark for 45 min at RT (25°C).
4. Discard solution, wash plate 5 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (250 µL) using a multi-channel pipette or auto washer. Let it soak for 1 min, and then remove all residual wash-liquid from the wells. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials.
5. Add 50 µl Substrate A solution, then add 50 µl Substrate B solution to each well, oscillate gently for 5s, incubate for 15 min at RT in dark.
6. Add 50 µL Stop Solution to each well and oscillate gently to stop the reaction.
7. Read result at 450 nm within 10 minutes.

Calculation

$$\boldsymbol{Percentage\ of\ Absorance\ Value}\ (\%) = \frac{\mathbf{A}}{\mathbf{AO}} \times \mathbf{100}\%$$

Where: **A** = Average (double wells) OD vale of sample or standard solution
AO = Average OD value of the 0 ppb standard solution

1. The standard curve can be plotted as the absorbance percentage of each standard solution (Y) vs. the corresponding log of each standard concentration (X).
2. The concentration of the samples can be interpolated from the standard curve.
3. Take the absorbance percentage of samples substitute into the standard curve, then get the corresponding concentration from the standard curve.
4. Multiplied by the corresponding dilution times = the actual concentration of Ciprofloxacin in samples.

Technical Support

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