Ciprofloxacin (Cipro) ELISA Kit

rev 05/20

(Catalog # E4365-100, 100 assays, Store at 4°C)

I. Introduction:

Ciprofloxacin is an antibiotic used to treat a number of bacterial infections. It is commonly used to treat acne and rosacea. Historically it was important in reducing the number of deaths from cholera. A broad-spectrum antibiotic of the polyketide class, it is produced by the actinobacterial genus Streptomyces. It acts by inhibiting protein synthesis. It is first-line therapy for rocky mountain spotted fever, Lyme disease, Q fever, psittacosis and lymphogranuloma venereum, mycoplasma pneumoniae and to eradicate nasal carriage of meningococci. **BioVision's Ciprofloxacin ELISA kit** is a competitive ELISA assay for the quantitative measurement of Ciprofloxacin in tissues, honey and urine. The density of color is proportional to the amount of Ciprofloxacin captured from the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Ciprofloxacin.
Detection Range: 0.1 – 8.1 ppb (ng/ml)
Sensitivity: < 0.1 ppb
Detection limit: 0.3 ppb for tissue, 0.4 ppb for honey, 3 ppb for milk and egg, 6 ppb for milk powder
Cross Reactivity: Sarafloxacin – 110%; Oxolin acid – 28%; Levofloxacin – 10%; Lomefloxacin, Marbofloxacin – 4%

III. Sample Type:

Tissue, honey, eggs, milk, milk powder

IV. Kit Contents:

Components	E4365-100	Part No.
Micro ELISA Plate	8 X 12 strips	E4365-100-1
Standard (S1 – S6)	1.0 ml X 6	E4365-100-2-x
High standard (100 ppb)	1.0 ml	E4365-100-3
Antibody working solution	5.5 ml	E4365-100-4
Enzyme conjugate	5.5 ml	E4365-100-5
Substrate A solution	6 ml	E4365-100-6
Substrate B solution	6 ml	E4365-100-7
Stop Solution	6 ml	E4365-100-8
Concentrated Wash Solution (20X)	40 ml	E4365-100-9
Concentrated Redissolving solution (5X)	50 ml	E4365-100-10
Plate Sealer	1	E4365-100-11

V. User Supplied Reagents and Equipment:

- 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

VI. Storage and Handling:

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

VII. Reagent Preparation:

Note: Prepare reagents within 30 minutes before the experiment.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **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

2. **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.
3. **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.
4. **Sample Extracting Solution**: Mix 10ml 0.15M HCl with 90 ml anhydrous acetonitrile, mix completely.
5. **Sample Preparation**:

Note: 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.

FOR RESEARCH USE ONLY! Not to be used on humans.

- **Tissue, liver, egg samples:** Weigh 2 g homogeneous sample into 50 ml centrifuge tube, Add 8ml sample extract solution, mix with vortex for 5 min , centrifuge at 4000 r/min at room temperature for 10 min. Take 2 ml clear organic phase of upper into the a 10 ml glass tube, and dry at 50 to 60°C with nitrogen or water bath. Add 1ml N-hexane, mix with vortex for 2 min, then add 1 ml redissolving solution, mix with vortex for 30seconds, centrifuge at 4000 r/min at room temperature for 5 min. Wipe out the upper N-hexane; take 50µl Lower water phase to be analyses. (Dilution factor: 2)
- **Honey**: Weigh 1.0 g homogeneous sample into 50ml centrifuge tube, add 6 ml sample extract solution, oscillate 5min to make it dissolve completely. Add 3 ml redissolving solution, then add 11 ml dichloromethane (CH_2CL_2), oscillate 5min, centrifuge at 4000 r/min at room temperature for 5 min. Wipe out the upper phase, take 8 ml organic phase to dry container, and dry at 50 to 60 ℃ with nitrogen or water bath. Dissolve the dry residue with 1 ml redissolving solution, then add 1ml N-hexane, mix 30s, centrifuge at 3000 r/min at room temperature for 5 min. Wipe out the upper phase; take 50µl Lower phase to be analyses. (Dilution factor: 2)
- **Milk Sample**: Take 25µl milk sample and 475µl redissolving solution, mix and oscillate 1min. Use 50µl solution to be analyses. (Dilution factor: 20)
- **Milk Powder**: Weigh 0.5 g homogeneous sample into 10ml centrifuge tube, add 5 ml deionized water and oscillate. Take 100µl sample and 400µl redissolving solution (Liquor 3), mix and oscillate 1min. Use 50µl solution to be analyses. (Dilution factor: 50)
- **Egg sample**: Weigh 1.0 g homogeneous sample into 10ml centrifuge tube, add 5 ml deionized water, oscillate and make it dissolves fully. Take 100µl sample and 400 µl redissolving solution, mix and oscillate 1min. Use 50µl solution for analysis. (Dilution factor: 30)

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. Shake the reagent bottles if there is any crystal. 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 autowasher. 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 50ul Substrate **A solution**, then add 50ul **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.

IX. CALCULATION:

Percentage of absorbance value (%) = A/A_0 X 100%

A: the average (double wells) OD value of the sample or the standard solution; A_0: the average OD value of the 0 ppb standard solution.

To draw the standard curve and calculate, take absorbance percentage of standards as Y-axis, the corresponding log of standards concentration (ppb) as X-axis. Draw the standard semilog curves with X-axis and Y-axis. Take absorbance percentage of samples substitute into standard curve, then can get the corresponding concentration from standard curve; last, Multiplied by the corresponding dilution times is the actual concentration of Sal of samples.

X. RELATED PRODUCTS:

- Salbutamol (SALB) ELISA Kit (Cat. No. K4209-100)
- Sulfonamides residue ELISA Kit (Cat. No. K4207-100)
- Aflatoxin B1 (AFB1) ELISA Kit (Cat. No. K4208-100)
- Fluoroquinolones ELISA Kit (Cat. No. K4205-100)
- Gentamicin ELISA Kit (Cat. No. K4206-100)
- Gentamicin (serum/urine) ELISA Kit (Cat. K4315-100)
- Kanamycin ELISA Kit (Cat. No. K4210-100)
- Streptomycin ELISA Kit (Cat. No. E4272-100)
- Melamine ELISA Kit (Cat. No. E4274-100)

155 S. Milpitas Blvd., Milpitas, CA 95035 USA | T: (408)493-1800 F: (408)493-1801 | www.biovision.com | tech@biovision.com