Chloramphenicol (CAP) ELISA Kit

rev 01/19

(Catalog # K4230-100, 100 assays, Store at 4°C)

I. Introduction:

Chloramphenicol is an antibiotic useful for the treatment of a number of bacterial infections. This includes meningitis, plague, cholera, and typhoid fever. Its use is only recommended when safer antibiotics cannot be used. Chloramphenicol may cause bone marrow suppression during treatment; this is a direct toxic effect of the drug on human mitochondria. BioVision's Chloramphenicol ELISA kit is a competitive ELISA assay for the quantitative measurement of human Chloramphenicol in animal tissue or food. The density of color is proportional to the amount of human Chloramphenicol captured from the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Chloramphenicol.
Detection Range: 0.025 – 2.025 ppb
Sensitivity: < 0.025 ppb
Detection Limit: 0.025ppb for tissue, liver, honey, milk; 0.05 ppb for urine, serum, casing, feed, milk powder; 0.1 ppb for eggs

III. Specificity:

Universal
Cross reaction rate: ＜0.1% for Thiamphenicol and Florfenicol

IV. Sample Type:

Tissue, Liver, Honey, Milk, Eggs, Urine, Serum, Feed, Milk powder

V. Kit Contents:

Components	K4230-100	Part No.
Micro ELISA Plate	8 X 12 strips	K4230-100-1
Standards (0, 0.025, 0.075, 0.225, 0.675, 2.025 ppb)	1 ml X 6	K4230-100-2
High standard (100 ppb)	1 ml	K4230-100-3
Antibody working solution	5.5 ml	K4230-100-4
Enzyme conjugate	11 ml	K4230-100-5
Substrate A solution	6 ml	K4230-100-6
Substrate B solution	6 ml	K4230-100-7
Stop Solution	6 ml	K4230-100-8
Concentrated Wash Solution (20X)	40 ml	K4230-100-9
Concentrated Redissolving solution (2X)	50 ml	K4230-100-10
Plate sealers	1	K4230-100-11

VI. User Supplied Reagents and Equipment:

- Reagents: Ethyl Acetate, N-hexane, acetonitrile, NaAc, acetic acid, $K_2Fe(CN)_5(NO)\cdot 2H_2O$, Glucuronidase , $ZnSO_4\cdot 7H_2O$
- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes and centrifuge tube for preparing standards or sample dilutions
- Absorbent paper

VII. Storage and Handling:

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

VIII. Reagent Preparation:

Note: Read the entire protocol before starting.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Wash Buffer:** Dilute 40 ml of the concentrated washing buffer with the distilled or deionized water to 800ml (or just to the required volume) for using.
2. **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.

- **Tissue, fish, shrimp, liver samples**: Weigh 3 g homogeneous samples into 50 ml centrifuge tube. mix with 3 ml ddH2O and vortex, then add 6 ml ethyl acetate, mix with vortex for 2min, centrifuge at 4000 rpm at RT (25°C) for 10 min. Transfer 2.5 ml supernatant to another centrifuge tube and blow dry at 50 to 60°C with nitrogen or air. Add 1 ml N-hexane to dissolve the dried residue, and then add 1 ml redissolving solution, oscillate 30s, centrifuge at 4000 rpm at room temperature for 5 min. Remove the upper N-hexane; take 50µl Lower water phase to be analyses. (Dilution Factor: 0.5)

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- **Serum, Plasma samples**: Take 1 ml sample into centrifuge tube, add 2 ml ethyl acetate, oscillate for 1 min, then centrifuge at 4000 rpm at RT for 5 min to separate water and organic phase. Transfer all supernatant to another centrifuge tube and blow dry at 50 to 60°C with nitrogen or air. Add 1ml N-hexane to dissolve the dried residue, and then add 1 ml redissolving solution, oscillate 30s, centrifuge at 4000 rpm at room temperature for 5 min. Remove the upper N-hexane; take 50µl Lower water phase to be analyses. (Dilution Factor: 1)
- **Urine samples**: Take 2 ml urine into centrifuge tube , add 0.5 ml of 0.1 M NaAc buffer (pH4.8), mix, then add 40 µl glucuronidase, mix, hydrolyze at 37°C for 2 hours or overnight. Return the solution to room temperature, add 8 ml ethyl acetate, oscillate for 1 min, and centrifuge at 4000 rpm at room temperature for 10 min. Transfer 4 ml supernatant to another centrifuge tube and blow dry at 50 to 60°C with nitrogen or air. Add 1 ml redissolving solution to dissolve the dried residue, mix. Use 50 µl for the assay. (Dilution Factor: 1)
- **Honey samples:** Weigh 2 g Homogeneous samples into centrifuge tube, add 4 ml deionized water to dissolve, and then add 4 ml ethyl acetate, oscillate 1 min, centrifuge at 4000 rpm at room temperature for 10min. Wipe out 2ml supernatant to another centrifuge tube and blow dry at 50 - 60˚C with nitrogen or air. Add 0.5 ml redissolving solution to dissolve the dried residue, mix. Use 50µl for the assay. (Dilution Factor: 0.5)
- **Milk samples:** Centrifuge the milk sample at 4000 rpm at 15°C for 10 min, wipe out the upper fat. Take 5 ml sample into 50 ml centrifuge tube, add 250 µl 0.36 M K2Fe(CN)5(NO)•2H2O solution (0.119 g/ml), and oscillate for 30 sec. Add 250 µl 1.04 M ZnSO4•7H2O solution (0.298 g/ml), oscillate for 30 sec, and centrifuge at 4000 rpm at 15°C for 10 min. Take upper liquid 2.2 ml (amount to 2 ml milk) into another centrifuge tube, add 4 ml ethyl acetate, mix and oscillate 2 min, centrifuge at 4000 rpm at room temperature for 10 min. Take 2 ml supernatant to blow dry at 50 - 60˚C with nitrogen or air. Add 0.5 ml redissolving solution to dissolve the dried residue, mix. Use 50 µl for the assay. (Dilution Factor: 0.5)
- **Milk powder samples**: Weigh 2 g milk powder into 50 ml centrifuge tube; dissolve with 10 ml deionized water. Add 1 ml 0.36 M $K_2Fe(CN)_5(NO)•2H_2O$ solution (0.119 g/ml),and add 1 ml 1.04 M $ZnSO_4•7H_2O$ solution (0.298 g/ml), oscillate and mix, then centrifuge at 4000 rpm at 15˚C for 10 min. Take upper liquid 3.6 ml into another centrifuge tube, add 4 ml ethyl acetate, mix and oscillate for 5 min, centrifuge at 4000 rpm at RT for 10 min. Take 4 ml supernatant to blow dry at 50 - 60˚C with nitrogen or air. Add 0.4 ml redissolving solution to dissolve the dried residue, mix. Use 50 µl for the assay. (Dilution Factor: 1)
- **Eggs sample**: Weigh 3 g homogeneous eggs sample into 50 ml centrifuge tube, add 9 ml acetonitrile-water solution (84%), oscillate for 2 min, then centrifuge at 4000 rpm at 15˚C for 10 min. Transfer 3 ml from upper liquid phase into another centrifuge tube, add 3 ml deionized water, then add 4.5 ml ethyl acetate, mix and oscillate 1 min, centrifuge at 4000 rpm at 15˚C for 10 min. Take all the supernatant to blow dry at 50 - 60 ˚C with nitrogen or air. Add 1ml N-hexane to dissolve the dried residues, then add 1 ml redissolving solution, oscillate 30s, centrifuge at 4000 rpm at RT for 5 min. Remove the upper N-hexane; take 50 µl Lower water phase to be analyses.
- **Feed**: Weigh 2 g homogeneous feed sample into 50 ml centrifuge tube, add 2 ml ddH2O, then add 6 ml ethyl acetate, oscillate for 2 min, centrifuge at 4000 rpm at 15˚C for 10 min. Transfer 3 ml from upper liquid phase into another centrifuge tube, blow dry at 50 - 60˚C with nitrogen or air. Add 1ml N-hexane to dissolve the dried residues, then add 1 ml redissolving solution, oscillate for 30 sec, centrifuge at 4000 rpm at room temperature for 5 min. Remove upper phase; take 50 µl from lower water phase for analysis.

IX. 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 VIII.
2. Add 50 µl **standards** or **samples** into marked well, then add 50 µl **antibody working solution** into each well. Oscillate the plate for 5 sec, cover the well and incubate for 30 min at RT (25°C).
3. 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.
4. Add 100 µl **Enzyme conjugate solution** into each well; avoid the light to incubate for 30 min at 25℃
5. Wash the plate with step 4.
6. Pipette 50 µl **Substrate A solution**, then pipette 50 µl **Substrate B solution** to each well, oscillate gently for 5 sec, avoid the light preservation for 15 min at RT (Do not put any substrate back to the original container to avoid any potential contamination.)
7. Add 50 µl **Stop Solution** to each well and oscillate gently to stop the reaction. Read result at 450 nm within 10 minutes.

X. 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 samples.

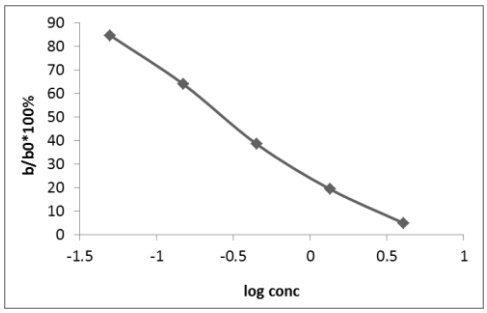

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

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