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# Chlorpromazine ELISA Kit

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**(Catalog # E4956-100, 96 assays, Store at 4°C)**

## I. Introduction:

Chlorpromazine is a phenothiazine that belongs to the class of antipsychotic drugs. It acts as a dopamine antagonist and possesses anti-serotonin and anti-histaminergic activity. It is generally used to treat psychotic disorders such as schizophrenia, bipolar disorder, and amphetamine-induced psychosis. It is also used to treat attention deficit hyperactivity disorder in children. In food-producing animals, this drug is used to calm animals. It is also used as feed additives to promote the growth of animals. However, daily use could lead to the accumulation of the drug in animal bodies which may adversely affect the circulatory and nervous system of animals. BioVision's Chlorpromazine ELISA kit is used to quantitatively measure Chlorpromazine in tissue samples. The kit is based on the Competitive ELISA principle. Samples and standards are added to the microwell plate that is pre-coated with an antigen and competes for binding to the anti-Chlorpromazine antibody. The HRP conjugate is added to each well and any unattached conjugates are washed off using Wash Buffer. The HRP enzymatic reaction is detected by the addition of substrate reagents. Finally, the reaction is terminated with an acidic stop solution. The color developed is inversely proportional to the concentration of Chlorpromazine in the samples.

## II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Chlorpromazine
Detection Limit: 0.5ppb for muscle
Sensitivity: 0.1ppb
Cross reaction: Chlorpromazine ---100%

## III. Sample Type:

Tissue (muscle)

## IV. Kit Contents:

| Components | E4956-100 | Part No. |
|---|---|---|
| Micro ELISA Plate | 8 X 12 Strips | E4956-100-1 |
| Standard (S0 – S4) | 1 ml X 5 | E4956-100-2 |
| HRP Conjugate | 12 ml | E4956-100-3 |
| Antibody working solution | 7 ml | E4956-100-4 |
| Substrate A | 6 ml | E4956-100-5 |
| Substrate B | 6 ml | E4956-100-6 |
| Stop Solution | 6 ml | E4956-100-7 |
| Wash Buffer (20X) | 25 ml | E4956-100-8 |
| Reconstitution Buffer | 50 ml | E4956-100-9 |
| Plate Sealer | 3 | E4956-100-10 |

## V. User Supplied Reagents and Equipment:

- Chemicals: deionized water, Acetone, n-Hexane, Trichloroacetic acid, NaOH
- Microplate reader, Nitrogen evaporator
- Clean eppendorf tubes and graduated cylinders 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. Opened kit may be stable for 1 month at 4°C.

## VII. Reagent and Sample Preparation:

Note: Bring all reagents to room temperature (20-25°C) 30 minutes before use.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Wash Buffer (1X):** Dilute 1 part of **Wash buffer (20X)** with 19 parts of deionized water. Prepare quantity as needed.

2. **Standards Preparation:** Ready-to-use standards provided as follows

| Standards | S0 | S1 | S2 | S3 | S4 |
|---|---|---|---|---|---|
| Conc. (ppb) | 0 | 0.1 | 0.3 | 0.9 | 2.7 |

3. **Sample Preparation:**
Note: The prepared sample maybe stored for up to one day at 2-8°C.

**Sample pre-treatment:** The following method must be used for pre-treatment of any kind of sample:
Note: Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents.

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

**Solution preparation before sample pre-treatment:**

1) **2.5% Trichloroacetic acid solution:** Weigh 2.5 grams of **Trichloroacetic acid** and make up the volume to 100 ml with deionized water. Mix well.
2) **3M NaOH solution:** Weigh 12 grams of **NaOH** and dissolve it in 100 ml with deionized water. Mix well.

**Sample Preparation and pre-treatment (for livestock muscle tissue samples):**

**Detection limit: 0.5ppb**

- Take 2 ± 0.05 grams of the homogenized tissue sample into a 50 ml centrifuge tube. Add 2 ml of **2.5% Trichloroacetic acid solution** and 6 ml of **Acetone**. Mix for 5 min. Centrifuge at 4000 r/min for 5 mins.
- Take 2 ml of the supernatant in a new tube and add 2 ml of **3M NaOH solution**, and then add 4.5 ml of **n-Hexane**. Mix for 5 mins. Centrifuge at 4000 r/min for 5 mins.
- Take 3 ml of the upper organic phase into a new centrifuge tube, blow-dry with nitrogen evaporator or water bath at 50 – 60 $^0$C.
- After blow-dry, add 0.5 ml of **Reconstitution Buffer** to dissolve the sample residue. Mix for 2 mins.
- Take 50 µl of the supernatant for the analysis.
- **Fold of dilution of the sample: 2**

## VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Add 50 µl of the **sample or standards** to separate duplicate wells. Add 50 µl of the **Antibody working solution** into each well. Mix gently for 5 secs by shaking the plate manually, seal the microplate with the plate sealer, and incubate in dark at 25 $^0$C for 30 minutes in the dark.
2. Remove the plate sealer carefully, aspirate liquid out of microwells, and add 300 µl of **Wash Buffer (1X)** to each well. Wash for 30 secs, and then discard the buffer. Repeat the washing step five times. After the final wash step, tap to dry (if there are the bubbles after tapping, remove them with the clean tips).
3. Add 100 µl of **HRP Conjugate** in each well and incubate at 25 $^0$C for 30 minutes in the dark.
4. Repeat washing as mentioned in **step 2**.
5. Add 50 µl of the **Substrate A** and then add 50 µl of the **Substrate B** into each well. Mix gently for 5 secs by shaking the plate manually, and incubate at 25 $^0$C for 15 mins in dark.
6. Add 50 µl of the **Stop Solution** into each well. Mix gently for 10 secs by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value (Recommend reading the OD value at the wavelength 450 nm within 5 mins).

## IX. Calculation:

- **Quantitative determination**

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

**Absorbance Value (%) = $B/B_0$ X 100%**

B: The average absorbance value of the sample or standard

$B_0$: The average absorbance value of the 0 ppb standard

To draw a standard curve: Plot the absorbance value of standards as y-axis, logarithmic of the concentration of the Ribavirin standards solution (ppb) as x-axis. The Ribavirin concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

## X. Related Products:

Sulfamethazine ELISA Kit (E4778)
Norfloxacin ELISA Kit (E4776)
Chlortetracycline ELISA Kit (E4782)
Ampicillin ELISA Kit (E4350)
Salbutamol (SALB) ELISA Kit (K4209)