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Amantadine ELISA Kit

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(Catalog # E4940-100, 96 assays, Store at 4°C)

I. Introduction:

Amantadine is an antiviral medication that is used to treat dyskinesia, a movement disorder caused by Parkinson's disease. Although the mechanism is not clearly understood, it is known to act as a dopamine agonist and antagonist for the NMDA receptor. Due to mild side effects on the central nervous system, it is recommended not to combine Amantadine with other CNS stimulants or anticholinergic drugs. Additionally, it is also used to treat influenza A infection. Amantadine blocks the influenza A M2 ion channel and thereby inhibits the replication of the virus. BioVision's Amantadine ELISA kit is used to quantitatively measure Amantadine in samples such as muscle tissue, milk, and eggs. 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-Amantadine 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 Amantadine in the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Amantadine
Detection limit: 1ppb for muscle, eggs, 2ppb for milk
Sensitivity: 0.5ppb
Cross reaction: Amantadine 100%, Rimantadine 230%, Somantadine 240%, Memantine < 1%

III. Sample Type:

Tissue (muscle), eggs, milk

IV. Kit Contents:

Components	E4940-100	Part No.
Micro ELISA Plate	8 X 12 Strips	E4940-100-1
Standard (S0 – S5)	1 ml X 6	E4940-100-2
HRP Conjugate	7 ml	E4940-100-3
Antibody working solution	7 ml	E4940-100-4
Substrate A	6 ml	E4940-100-5
Substrate B	6 ml	E4940-100-6
Stop Solution	6 ml	E4940-100-7
Wash Buffer (20X)	25 ml	E4940-100-8
Sample Solution (20X)	10 ml	E4940-100-9
Plate Sealer	3	E4940-100-10

V. User Supplied Reagents and Equipment:

- Chemicals: deionized water, Acetonitrile, NaCl, $ZnSO_4.7H_2O$
- 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. The 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.

2. **Ready to use Standards Concentration:**

Standards	S0	S1	S2	S3	S4	S5
concentration (ppb)	0	0.5	1.5	4.5	13.5	40.5

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) **1M $ZnSO_4$ Solution:** Mix 2.88 grams of **$ZnSO_4.7H_2O$** with 8.64 ml of deionized water to prepare **1M $ZnSO_4$ Solution**. **Please note, 1M ZnSO4 Solution is unstable, hence a fresh solution needs to be prepared before starting the experiment.**
2) **Sample Solution (1X):** Dilute 1 part of **Sample Solution (20X)** in 19 parts of deionized water

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

Detection limit: 1ppb

- Take 2 ± 0.05 g of the homogenized tissue sample into 50 ml centrifuge tube, add 0.5 grams of **NaCl**, and then add 6 ml of **Acetonitrile**. Mix for 1 min and centrifuge at 4000 r/min for 5 mins.
- Take 4 ml of supernatant in a new tube and dry with nitrogen evaporator or water bath at 50 – 60 ^{0}C.
- After drying the sample, add 0.5 ml **Sample solution (1X)**, mix for 30 sec. Take 50 µl for detection
- **Dilution factor of the sample: 0.5**

Sample Preparation and pre-treatment (for egg samples):

Detection limit: 1ppb

- Take 2 ± 0.05 g of the homogenized egg sample into 50 ml centrifuge tube, add 6 ml of **Acetonitrile**. Mix for 1 min and centrifuge at 4000 r/min for 5 mins.
- Take 4 ml of supernatant in a new tube and dry with nitrogen evaporator or water bath at 50 – 60 ^{0}C.
- After drying the sample, add 0.5 ml **Sample solution (1X)**, mix for 10 sec. Take 50 µl for detection
- **Dilution factor of the sample: 0.5**

Sample Preparation and pre-treatment (for milk samples):

Detection limit: 2ppb

- Take 0.5 ml of milk sample into 4 ml centrifuge tube; add 50 µl of **1M $ZnSO_4$ Solution**.
- Add 2 ml of **Sample Solution (1X)**, mix for 10 sec, centrifuge at 4000 r/min for 5 mins. Take 50 µl for detection.
- **Dilution factor of the sample: 4**

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 **HRP Conjugate**, then 50 µl of the **Antibody working solution** into each well. Mix gently for 10 sec, seal the microplate with the plate sealer, and incubate at 25 ^{0}C for 30 minutes in dark.
2. Remove the plate sealer carefully, aspirate liquid out of microwells, and add 260 µl of **Wash Buffer (1X)** to each well. Wash for 30 secs, and then discard the buffer. Repeat the washing step four times. After the final wash step, tap to dry (if there are the bubbles after tapping, remove them with the clean tips).
3. Add 50 µl of the **Substrate A** and then 50 µl of the **Substrate B** into each well. Mix gently by shaking the plate manually, and incubate at 25 ^{0}C for 15 mins at dark.
4. Add 50 µl of the **Stop Solution** into each well. Mix gently 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 (%) = A/A_0 X 100%

A: The average absorbance value of the sample or standard

A_0: The average absorbance value of the 0 ppb standard

To draw a standard curve: Take the absorbency value of standards as y-axis, logarithmic of the concentration of the Amantadine standards solution (ppb) as x-axis. The Amantadine 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:

Sulfadiazine ELISA Kit (E4904)
Diethylstilbestrol (DES) ELISA Kit (E4278)
Ractopamine ELISA Kit (E4565)
Ciprofloxacin (Cipro) ELISA Kit (E4365)
Tylosin ELISA Kit (E4779)