Elabscience®

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Am (Amantadine) ELISA Kit

Catalog No: E-FS-E006

96T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

This kit uses Indirect-Competitive-ELISA as the method. It can detect tissue, such as beef, chicken, pork, etc. This kit is composed of Micro ELISA Plate, HRP conjugate, antibody, standard and other supplementary reagents. The microplate provided in this kit has been pre-coated with Am (Amantadine). During the test, Am in the samples or standard competes with Amantadine on the solid phase supporter for sites of Am antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is for color development. There is a negative correlation between the OD value of samples and the concentration of Am. The concentration of Am in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity: 0.5 ppb (ng/mL)

Reaction mode: 25℃, 30 min~ 30 min~ 15 min

Detection limit: Tissue (beef, chicken, pork) ---0.5 ppb,

Cross-reactivity: Amantadine---100%, Amoxicillin---＜0.1%,

Ceftiofur---＜0.1%,

Sample recovery rate: Tissue---90%±20%

Kits components

Item	Specifications
Micro ELISA Plate	96 wells
Standard Liquid	1 mL each (0 ppb, 0.5 ppb, 1.5 ppb, 4.5 ppb, 13.5 ppb, 40.5 ppb)
High Concentrated Standard (100 ppb)	1 mL
HRP Conjugate (red cap)	11 mL
Antibody Working Solution (blue cap)	5.5 mL
Substrate Reagent A (white cap)	6 mL
Substrate Reagent B (black cap)	6 mL
Stop Solution (yellow cap)	6 mL
20×Concentrated Wash Buffer (white cap)	40 mL
2×Reconstitution Buffer (yellow cap)	50 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Other supplies required

Instruments: Micro-plate reader, Printer, Homogenizer, Nitrogen Evaporators, Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01 g).

High-precision transferpettor: single channel (20-200 μL, 100-1000 μL), Multichannel (300 μL).

Reagents: Acetonitrile**,** Deionized water, sodium sulphate anhydrous.

Experimental preparation

Bring all reagents and samples to room temperature before use.

Open the micro-plate reader in advance, preheat the instrument, and set the testing parameters.

1. **Sample pretreatment Notice:**

   Experimental apparatus must be clean, and the pipette should be disposable to avoid cross-contamination during the experiment.

2. **Solution preparation**

   Solution 1**:** Reconstitution buffer

   Double dilute the 2×Reconstitution buffer with deionized water.

   (2×Reconstitution buffer (V): Deionized water (V) = 1:1). Reconstitution buffer can be stored for 1 month at 4 degrees.

   Solution 2: Wash Buffer

   Dilute 20×Concentrated Wash Buffer with deionized water. (20× Concentrated Wash Buffer (V): Deionized water (V) = 1:19)

3. Sample pretreatment procedure

3.1 Pretreatment of tissue:

(1) Weigh 3±0.05 g of homogeneous tissue, add 6 mL of acetonitrile, 1 g of sodium sulphate anhydrous. And then oscillate fully for 3 min, centrifuge at 4000 r/min at room temperature for 10 min.

(2) Take 2 mL of supernatant, blow-dry in 50-60℃ nitrogen or air.

(3) Add 0.5 mL of 1×Reconstitution buffer and 0.5 mL n-hexane, then oscillate for 30 sec, centrifuge at 4000 r/min for 5 min, and then remove upper layer liquid, take the lower layer liquid 100 μL for analysis.

Note: Sample dilution factor: 0.5, Minimum detection dose: 0.5 ppb

Assay procedure

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Number:** number the samples and standard in order (multiple well), and keep a record of standard wells and sample wells.
2. **Add sample:** add 100 μL of Standard or Samples per well, then add 50 μL of Antibody Working Solution, oscillate gently for 5 sec to mix thoroughly, react for 30 min avoiding light at 25℃.
3. **Wash**: remove the liquid in the wells, then add 250 μL of wash buffer to each well and wash. Repeat wash procedure for 5 times, 30 sec intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **HRP conjugate**: add 100 μL of HRP Conjugate to each well, incubate for 30 min at 25℃ in the dark.
5. **Wash**: Repeat step 3.
6. **Color Development:** add 50 μL of substrate solution A to each well, and then add 50 μL of substrate solution B. Gently oscillate for 5 sec to mix thoroughly. Incubate shading light for 15 min at 25℃ (The reaction time can be extended according to the actual color change).
7. **Stop reaction:** add 50 μL of stop solution to each well, oscillate gently to mix thoroughly to stop the reaction.
8. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm with a micro-plate reader (the 450/630 nm double wavelength is recommended).This step should be finished in 10 min after stop reaction.

Result analysis

1. **Absorbance (%) = $A/A_0 \times 100\%$**

   A: Average absorbance of standard or sample

   A_0: Average absorbance of 0 ppb Standard

2. **Drawing and calculation of standard curve**

   Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add average absorbance value of sample to standard curve to get corresponding concentration. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

   For this kit, it is more convenient to use professional analysis software for accurate and fast analysis on a large number of samples.

Notes

1. Overall OD value will be lower when reagents is not brought to room temperature before use or room temperature is below 25℃.
2. During the washing procedure, if the wells turn dry, it will lead to bad linear standard curve and poor repeatability, move on to the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the reproducibility of this ELISA kit.
4. Micro ELISA plate should be covered by plate sealer. Avoid the reagents to strong light.
5. Do not use expired kit and reagents of different batches of kits.
6. TMB should be abandoned if it turns color. When OD value of standard(concentration: 0) is below0.5 unit(A_{450nm}＜0.5), it indicates reagent is deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.

Storage and valid period

Storage: Store at 2-8℃. Avoid freeze / thaw cycles.

Valid Period: 1 year, production date is on the packing box.