VERSION 1.01

AMANTADINE ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Amantadine ELISA Test Kit

Catalogue Number. IP100050

Principle

This test kit is based on the competitive enzyme immunoassay for the detection of Amantadine. The coupling antigen is pre-coated on the micro-well stripes. The Amantadine in the sample and the coupling antigens pre-coated on the micro-well stripes compete for the anti- Amantadine antibodies. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the sample has a negative correlation with the Amantadine in the sample. This value is compared to the standard curve and the Amantadine residues is subsequently obtained.

Technical specifications

Sensitivity: 0.2ppb
Incubator temperature: 25℃
Incubator time: 30min～15min
Detection limit: Chicken, duck.0.2ppb
Cross-reaction rate: Amantadine 100%
Recovery rate: Chicken, duck90±25%

Components

1	Micro-well strips	12 strips with 8 removable wells each	
2	6× standard solution (1mL each)	0 ppb, 0.2 ppb, 0.8 ppb, 3.2 ppb, 12.8 ppb, 51.2 ppb	
3	Enzyme conjugate	7ml	red cap
4	Antibody working solution	7ml	blue cap
5	Substrate A	7ml	white cap
6	Substrate B	7ml	black cap
7	Stop solution	7ml	yellow cap
8	Extractant	50ml*2	transparent cap
9	20× concentrated washing buffer	15ml	white cap
10	5× concentrated redissolving solution	10ml	white cap

Materials required but not provided

Equipment: ELISA Reader (450 nm/630nm), homogenizer, shaker, centrifuge, balance: 0.01g quantity sensitive, nitrogen-drying device, incubator, graduated pipettes, printer
Micropipettes: single-channel 20ml ~ 200ml, 100ml ~ 1000ml, multi-channel 30～300 µl
Reagents: Acetonitrile, n-hexane.

Sample pre-treatment

Instructions

1) Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents;

2) Before the experiment, each experimental utensil must be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.
Solution preparation before sample pre-treatment:
1) Sample extract solution
6 parts Acetonitrile + 1 parts Extractant to obtain the ready to use sample extract solution.
2) Sample redissolving solution
Use 1 part of concentrated redissolving solution (5X) and dissolve with 4 parts of deionized water to obtain the ready to use sample redissolving solution.

Samples preparation

a) Preparation of Chicken, duck sample

1) Take 3.0±0.05g homogenized tissue sample into 50ml centrifuge tube; add 6ml sample extract solution, shake for 3min, centrifuge at above 4000r/min at room temperature (20 - 25 ℃) for 5 min;
2) Take 2ml clear organic phase into a dry container, blow to dry with nitrogen or air at 50~60 ℃;
3) Firstly add 1ml n-hexane, then add 0.5ml Sample redissolving solution, mix for 30s, centrifuge at above 4000r/min at room temperature (20 - 25 ℃) for 5 min, discard up-layer n-hexane;
4) Take down-layer liquid 50µl to test
Dilution factor: 0.5

ELISA procedures

Instructions

1. Bring ELISA reagents to room temperature (20 - 25 °C) before use.
2. Put ELISA reagents back to 2-8 ℃ immediately after use
3. The ELISA reproducibility in the analysis process is largely depends on the consistency of the washing plate, the correct washing plate operation is the point of determination ELISA program
4. In all process of constant temperature incubation, avoid light exposure, seal the microplate with the cover membrane

Operation procedures

1. Bring test kit to the room temperature (20-25 ℃) for at least 30 min, note that each reagent must be shaken evenly before use;
2. Put the required micro-well strips into plate frames. Re-sealed the unused microplate, stored at 2-8 ℃, not frozen.
3. Solution preparation: dilute the 15ml 20× concentrated washing buffer with deionized water to 300ml.
4. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate; record their positions.
5. Add standard/sample: Add 50 µL of the sample or the standard solution into separate duplicate wells, then add enzyme conjugate, 50 µL/well; then antibody working solution, 50 µL/well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, incubate at 25 °C for 30 min in the dark.
6. Wash microplate: Carefully open the cover membrance, pour liquid out of microwell; add 250 µL/well of diluted washing buffer, wash fully for 4-5 times, 15-30 s each time, then take out and flap to dry with absorbent paper.(Use unused spear to pierce bubble after dry)
7. Coloration: add 50 µL of substrate A solution then 50 µL B solution into each well. Mix gently by shaking the plate manually, and incubate at 25 °C for 15min in the dark for coloration.

8. Determination: 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 450nm to determine the OD value of every well. (Recommend to read the OD value at the dual-wavelength 450/630nm within 5 min).

Result judgment

There are two methods to judge the results; the first one is the rough judgment, while the second is the quantitative determination. Note that the OD value of the sample has a negative correlation with the Amantadine in the sample

Qualitative determination

The concentration range (ng/ml) can be obtained by compared the average absorbance value with standards. Suppose absorbance value of Sample One is 0.3, Sample Two is 1.0, and the standards are: 0ppb of 2.243; 0.2ppb of 2.054; 0.8ppb of 1.715; 3.2ppb of 1.074; 12.8ppb of 0.451; 51.2ppb of 0.155. Then the concentration of the sample one is in the range of 12.8ppb ~ 51.2ppb; Sample Two is 3.2ppb ~ 12.8ppb. The concentration range of Amantadine in the samples can be obtained by multiplied by the corresponding dilution of the sample.

Quantitative determination

In order to calculate the concentration of samples, a standard curve should be made. Before standard curve is made, the concept of % absorbance should be know.
Calculation of % absorbance:
Percentage of absorbance value = $\frac{B}{B_0} \times 100\%$
B—the average OD value of the sample or the standard solution
B_0—the average OD value of the 0 ng/mL standard solution
The zero standard is thus made equal to 100 % and the absorbance values are quoted in percentages. The values calculated for the standards are entered in a system of coordinates on semilogarithmic graph paper against the the Amantadine concentration [ng/mL]. The Amantadine concentration in ng/mL (ppb) corresponding to the absorbance of each sample can be read from the calibration curve.
A special software for result analysis of ELISA will facilitate double or multiple determinations. If you need, please call to request.

Precautions

1. The room temperature below 25 ℃ or the temperature of the reagents and the samples being not returned to the room temperature (20-25 ℃) will lead to a lower standard OD value.
2. Dryness of the microplate in the washing process will be accompanied by the situations including the non-linear standard curves and the undesirable reproducibility; So continue to next step immediately after washing.
3. Mix evenly before adding any reagents.
4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.
5. Do not use the kit exceeding its expiry date. The use of diluted or adulterated reagents from the kits will lead to the changes in the sensitivity and the detecting OD values. Do not exchange the reagents from the kits of different lot numbers to use.
6. Storage: store at 2-8 ℃, not frozen. Put the unused microplate into an auto-sealing bag to re-seal it. The standard substance and the colourless color former is light sensitive, and thus they cannot be directly exposed to the light.

7. Discard the colouration solution with any color that indicates the degeneration of this solution. The detecting value(450/630nm) of the 0 standard solution (0 ppb) of less than 0.5(（A450nm<0.5) indicates its degeneration.
8. The optimum reaction temperature is 25 ℃, and too high or too low temperatures will result in the changes in the detecting sensitivity and OD values.

Storage: store at 2-8 ℃, not frozen.
Expiry date: 12 months; date of production is on box.