VERSION 1.01

CYPROHEPTADINE ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Cyproheptadine ELISA Test Kit

Catalogue Number. IP100044

Principle

This test kit is based on the competitive enzyme immunoassay for the detection of Cyproheaptadine in urine, tissue sample. The coupling antigens are pre-coated on the micro-well stripes. The Cyproheptadine in the sample and the coupling antigens pre-coated on the micro-well stripes compete for the anti- Cyproheptadine antibody, add TMB substrate for coloration. The higher Cyproheptadine in the sample, the less ELISA antibody combined at the solid phase, the lighter of the coloration. The optical density (OD) value of the sample has a negative correlation with the Cyproheptadine in it. This value is compared to the standard curve and the Cyproheptadine concentration is subsequently obtained.

Technical specifications

Sensitivity: 0.04 ppb
Incubation Temperature: 25 ℃
Incubation Time: 30min—15min
Detection limit: Urine 0.04 ppb, Tissue 0.08 ppb, Feed 0.4 ppb
Cross-reaction rate:Cyproheptadine 100%
Recovery rate: Urine 95±20%. Tissue 85±25%, Feed 85±25%

Components

1	Micro-well strips	12 strips with 8 removable wells each	
2	6× standard solution (1mL each)	0 ppb, 0.04 ppb, 0.12ppb, 0.36ppb, 1.08ppb, 3.24ppb	
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	20× concentrated washing buffer	40ml	white cap

Materials required but not provided

Equipments: microplate reader, printer, homogeniser, nitrogen-drying device, vortex, centrifuge, measuring pipets, and balance (a sensibility reciprocal of 0.01 g);
Micropipettors: single-channel 20-200 µL, 100-1000 µL, and multi-channel 30-300 µl;
Reagents: Acetonitrile, CH_2Cl_2, glacial acetic acid

Sample pre-treatment

Instructions

The following points must be dealt with before the pre-treatment of any kind of sample:

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 equipment 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) Acetonitrile-CH_2Cl_2 Solution: Mix 80ml Acetonitrile and 20ml CH_2Cl_2 evenly.
2) Sample extracting solution: Add 1ml glacial acetic acid into 100ml Acetonitrile-CH_2Cl_2 Solution

Samples preparation

a) Urine

Directly take 50µL bright urine to test (If the urine is turbid, it must be filtered or centrifuged for 10 mins at 4000r/min until bright urine obtained), the un-used urine should be frozen stored.
Fold of dilution of the sample: 1

b) Tissue

1) Weigh 2±0.05g homogenized tissue sample into 50ml centrifuge tube, add 8ml Sample extracting solution, shake fully for 3min, centrifuge at 4000r/min at 20℃ for 10min;
2) Take 2ml supernatant, blow to dry by nitrogen or air at 56℃, add 1ml N-hexane to dissolve the dry residue, then add 1ml deionized water , shake for 30s, centrifuge at 4000r/min at 20℃ for 10min, discard the up-layer organic phase.
3) Take 50µL down-layer to test.
Fold of dilution of the sample: 2

c) Feed

1) Grind sample, weigh 1.0±0.05 g into 50ml centrifuge tube, add 10 mL Sample extracting solution, shake fully for 3 min, centrifuge at above 4000 r/min at 20 ℃ for 10 min.
2) Take 1 ml supernatant, blow to dry with nitrogen or air at 56 ℃, use 1ml deionized water to dissolve the dry residue, shake fully for 90s;
3) Take 50 µL for analysis.
Fold of dilution of sample: 10

ELISA procedures

Instructions

1. Bring all reagents and micro-well strips to balance at the room temperature (20-25 ℃) before use.
2. Return all reagents to 2-8 ℃ immediately after use.
3. The reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing. The correct operation of plate washing is the key point in the procedures of ELISA.
4. For the incubation at constant temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.

Operation procedures

1. Bring test kits to room temperature (20-25 ℃) for at least 30 min. Note that each reagent must be shaken to mix evenly before use;

2. Put the required micro-well strips into plate frames. Re-sealed the unused microplate, store at 2-8 ℃, not frozen.
3. Solution preparation: Dilute 40ml (20×) concentrated washing buffer with deionized water at 1:19 (1 part concentrated washing buffer + 19 parts deionized water), or making solution as needed.
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 50 µL of the sample or standard solution to separate duplicate wells, add 50 µL Enzyme Conjugate into each well, then add 50ul antibody working solution, shake to even, and then seal the microplate with the cover membrane, and incubate at 25 ℃ for 30 min.
6. Pour liquid out of the wells, flap to dry on absorbent paper, add 250 µL/well of washing buffer to wash microplate for 15-30 sec, then take out and flap to dry with absorbent paper, repeat 4-5 times. (Use clean spearhead to puncture the bubbles)
7. Coloration: add 50 µL of the substrate A solution and then 50 µL of the B solution into each well. Mix gently by shaking the plate manually, and incubate at 25 ℃ for 15 min at 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 450 nm to determine the OD value of every well in 5 mins(Recommend to read the OD value at the dual-wavelength 450/630 nm).

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 content of Cyproheptadine.

Qualitative determination

The concentration range (ng/mL) can be obtained from comparing the average OD value of the sample with that of the standard solution. Assuming that the OD value of the sample Ⅰ is 0.3, and that of the sample Ⅱ is 1.0ppb, the OD value of standard solutions is: 2.243for 0 ppb, 1.816 for 0.04 ppb, 1.415 for 0.12 ppb, 0.74 for 0.36 ppb, 0.313 for 1.08 ppb, 0.155 for 3.24 ppb, accordingly the concentration range of the sample Ⅰ is 1.08 to 3.24 ppb, and that of the sample Ⅱ is 0.12 to 0.36 ppb. Multiply the corresponding dilution folder, then the actual concentration of Cyproheptadine is obtained.

Quantitative determination

The mean values of the absorbance values obtained for the percentage of the average OD value (B) of the sample and the standard solution divided by the OD value (B0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is,

Percentage of absorbance value = $(B / B_0) \times 100\%$

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
Draw the standard curve with the absorption percentages of the standard solution and the semilogarithm values of the Cyproheptadine standard solution (ng/mL) as Y- and X-axis, respectively. Read the corresponding concentration of the sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the corresponding dilution fold, finally obtaining the Cyproheptadine concentration in the sample.
Using the professional analyzing software of this kit will be more convenient for the accurate and rapid analysis of a large amount of samples. (Please contact us for this software).

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, otherwise there will be the undesirable reproducibility.
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. Discard the colouration solution with any color that indicates the degeneration of this solution. The detecting value of the standard solution 1（0 ppb）of less than 0.5 indicates its degeneration.
7. 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.