Clonidine ELISA Test Kit

Catalog No. LSY-10039

1. Principle

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

2. Technical specifications

Sensitivity: 0.05 ppb

Incubation Temperature: 25 ℃

Incubation Time: 30min—（10-15）min

Detection limit

Urine 0.05 ppb

Tissue (Method 1) 0.2 ppb

Tissue (Method 2) 0.1 ppb

Feed 0.5ppb

Cross-reaction rate

Clonidine 100%

Romifidine < 44%

Tizanidine < 8%

Xylazine < 0.03%

Recovery rate

Urine 90±10%

Tissue 90±15%

Feed 90±15%

3. Components

1) Micro-well strips: 12 strips with 8 removable wells each

2) 6× standard solution (1 mL each): 0 ppb, 0.05 ppb, 0.15ppb, 0.45 ppb, 1.35 ppb and 4.05 ppb

3) Enzyme lyophilized powder (2 tube) red cap

4) Enzyme diluent solution (12 mL) blue cap

5) Substrate A solution (7 mL) white cap

6) Substrate B solution (7 mL) black cap

7) Stop solution (7 mL) yellow cap

8) 20× concentrated washing buffer (40 mL) white cap

4. Materials required but not provided

1） **Equipments:** microplate reader, printer, homogeniser, nitrogen-drying device, vortex, centrifuge, measuring pipets, and balance (a sensibility reciprocal of 0.01 g);

2） **Micropipettors:** single-channel 20-200 µL, 100-1000 µL, and multi-channel 250 µL;

3） **Reagents:** n-Hexane, Acetonitrile, Methanol, Anhydrous Sodium Sulfate.

5. 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. .

5.1 Samples preparation

a) Urine

Directly take 20µL bright urine to test (If the urine is turbid, it must be filtered or centrifuged for 5 mins at 4000r/min until bright urine obtained), the un-used urine should be frozen stored.

Fold of dilution of the sample: 1 Quantitative detection limit: 0.1 ppb

(Consider the certain interference caused by impurities and individual difference of urine, so the detection limit of urine is 0.1 ppb)

b) Tissue (Meat, liver) Method No.1

1. Weigh 2± 0.05 g tissue, add 6 mL diluted sample extraction solution, shake thoroughly for 2 mins, centrifuge at above 4000 r/min at room temperature (20-25 ℃) for 10 min(If the fat content is high in the sample, incubate at 85℃ in water bath for 10 mins after shaking, then centrifuge).

2. Take 20 µL supernatant for analysis.

Fold of dilution of the sample: 4 Quantitative detection limit: 0.2 ppb

c) Tissue (Meat, liver) Method No.2

1. weigh 2± 0.05 g of the homogenized sample, add 8 ml Acetonitrile solution,shake thoroughly for 2 mins. Centrifuge at above 4000 r/min at 15 ℃ for 10 min.

2. Take 5ml supernatant, blow to dryness by nitrogen or air at 56 ℃.

Meat: Add 1 mL deionized water, mix and shake for 30s. Take 20 µL for analysis

Fold of dilution of the sample: 1 Quantitative detection limit: 0.05 ppb

3. **Liver:** Add 2 mL N-hexane, shake and dissolve. Then add 1 mL deionized water, mix and shake for 30s. Centrifuge at above 4000 r/min at 15 ℃ for 5 min. Remove the upper layer. Take 50µL lower layer, mix with 50µL deionized water evenly. Take 20 µL for analysis.

Fold of dilution of the sample: 2 Quantitative detection limit: 0.1 ppb

d) Feed

1. Weigh 1.0±0.05g pestle feed, add 10 ml Methanol, then add 5g Anhydrous Sodium Sulfate, shake on vortex for 2 mins.

2. Centrifuge at above 4000 r/min at 15 ℃ for 10 min.

3. Absorb 1ml supernatant after centrifuge, blow to dryness by nitrogen at 56 ℃. Dissolve the dry residue with 1 ml deionized water, then add 1 ml N-hexane, mix for 30s, centrifuge at above 4000 r/min at 15 ℃ for 5 min.

4. Take 20 µL lower layer for analysis.

Fold of dilution of the sample: 10 Quantitative detection limit: 0.5 ppb

6. ELISA procedures

6.1 Precautions

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.

6.2 Operation

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. **Preparation of enzyme concentrate: Add 100ul Enzyme diluent solution to each Enzyme lyophilized powder tube, tighten the lid until enzyme lyophilized powder is fully dissolved evenly, ready to use. If not use, store at 4℃ for 14 days.**

3. Put the required micro-well strips into plate frames. Re-sealed the unused microplate, store at 2-8 ℃, not frozen.

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. **Enzyme working solution preparation: dilute the dissolved enzyme concentrate with the enzyme diluent at 1:99. Make it according to the volume needed, the working solution can not be stored for a long time. (Enzyme concentrate is the solution of Enzyme lyophilized powder dissolved by 100ul enzyme diluent)**

6. Add 20 μL of the sample or standard solution to separate duplicate wells

7. Add 100 µL of the ready-to-use enzyme working solution into each well, then seal the microplate with the cover membrane, and **incubate at 25 ℃ for 30 min**.

8. 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)

9. 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 10-15 min** at dark for coloration;

10. 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).

7. 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 Clonidine.

.**7.1 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 0.311, and that of the sample Ⅱ is 0.715ppb, the OD value of standard solutions is: 1.642 for 0 ppb, 1.200 for 0.05 ppb, 0.780 for 0.15 ppb, 0.454 for 0.45 ppb, 0.236 for 1.35 ppb, 0.142 for 4.05 ppb, accordingly the concentration range of the sample Ⅰ is 1.35 to 4.05 ppb, and that of the sample Ⅱ is 0.15 to 0.45 ppb. Multiply the corresponding dilution folder, then the actual concentration of Clonidine is obtained.

7.2 Quantitative determination

The mean values of the absorbance values is obtained for 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 ×100%

B_0

B—the average (double wells) OD value of the sample or the standard solution

B_0—the average OD value of the 0ng/mL standard solution

Draw the standard curve with the absorption percentages of the standard solution and the semilogarithm values of the Clonidine 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 Clonidine 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) .

8. Precautions

1 The room temperature below 20 ℃ 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.

9. Storage and expiry date

Storage: store at 2 to 8 ℃, not frozen.

Expiry date: 12 months; date of production is on box.

Remarks: If the vacuum package of microtiter plates has leakage, the microtiter plate is normal and effective, do not affect the experimental result. Please feel free to use.

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