Malachite green ELISA kit

1. Principle

This test kit is based on the competitive enzyme immunoassay. The coupling antigen is pre-coated on the micro-well stripes. The Malachite green in the sample and the coupling antigens pre-coated on the micro-well stripes compete for the anti-Malachite green 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 Malachite green in it. This value is compared to the standard curve and the content of Malachite green residues is subsequently obtained.

2. Technical specifications

Sensitivity: 0.025 ppb

Detection limit

Shrimp, Fish, Water..... 0.1 ppb

Recovery rate

Shrimp, Fish ..... 75±10%

Cross-reaction rate

Malachite green..... 100%

Crystal violet..... 95%

Invisible malachite green..... 0.1%

Recessive crystal violet ……0.1%

3. Components

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

2） 100ppb Standard concentrated solution (1 mL)

3） Enzyme conjugate (12 mL) .....red cap

4） Antibody working solution (7 mL) .....red 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

9） A solution(50ml).....transparent cap

10） B solution(15ml).....blue cap

4. Materials required but not provided

(1) Equipments: microplate reader, homogenizer, nitrogen-drying device, vortex, oscillator, centrifuge, measuring pipets, balance (a sensibility reciprocal of 0.01 g).

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

(3)Reagents: Acetonitrile (CH_3CN), Methanol, deionized water, Concentrated HCL,NaCL, Dichloromethane(CH2CL2)

5. Sample pre-treatment

Instructions (The following points must be dealt with before the pre-treatment)

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(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. Acetonitrile (CH_3CN)-HCL solution:0.8ml Concentrated HCL dissolve in 1L Acetonitrile (CH_3CN)

2.20%NaCl solution: 25g NaCl dissolve in the 100ml deionized water

3.Diluted A solution:50ml A solution dissolve in the 50ml deionized water at V/V=1:1

4.Sample diluent:3ml Methanol dissolve in 28ml Diluted A solution at V/V=3:28(for redissolving sample and diluting standard solution.(On-demand preparation, are now equipped with)

Samples preparation(fish,shrimp,water)

(1) take 3±0.05g or 3ml water sample into 50ml centrifuge tube,add 3ml 20% NaCl solution,shake for 2min,add 8ml Acetonitrile (CH_3CN)-HCL solution,shake for 3min,add 4ml (CH2CL2),shake for 5 min, centrifuge at above 4000 r/min at 15 ℃ for 10 min,

(2)Take 3ml upper layer, blow to dry with nitrogen at 56℃, add 500uL of Sample diluent and 500uL B solution to redissolve,shake for 30s,place it for 10-15min

4 Take 50 μL for further analysis.

Fold of dilution of the sample: **Detection limit: 0.05 ppb**

6. Enzyme conjugate pre-treatment

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.

ELISA 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.Standard sample preparation

Take some quantity of 100ppb Standard concentrated solution dissolve in sample diluent,shake evenly.(Diluted with glass bottles are now available)

(1)2.025ppb Standard solution No.6:

40.5ul of 100ppb Standard concentrated solution dissolve in 1959.5ul sample diluent,mix up

(2)0.675ppb Standard solution No.5:

300ul of Standard solution No.6 dissolve in 600ul sample diluent,mix up

(3)0.225ppb Standard solution No.4:

300ul of Standard solution No.5 dissolved in 600ul sample diluent,mix up

(4)0.075ppb Standard solution No.3:

300ul of Standard solution No.4 dissolved in 600ul sample diluent,mix up

(5)0.025ppb Standard solution No.2:

300ul of Standard solution No.3 dissolved in 600ul sample diluent,mix up

(6)0ppb Standard solution No.1:

sample diluent

3.Take the required micro-well strips and plate frames. Re-sealed the unused microplate, stored at 2- 8 ℃.

4.Solution preparation: dilute 40 mL of the 20 × concentrated washing buffer with the distilled or deionized water at 1:19 to 800 mL (or just to the required volume) for use.

5.Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate; record their positions.

6.Add 50 µL of the sample and 50µL of the standard solution into each well, then add antibody working solution 50 µL/well, seal the microplate with the cover membrane, and incubate at 25 ℃ for 30 min.

7.Pour the liquid out of microwell, wash the microplate with the washing buffer at 250 µL/well for 4-5 times, each time for 15-30s, flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips).

8.Add 100 µL enzyme conjugate into each well, seal the microplate with the cover membrane,and incubate at 25 ℃for 30 min, continue as described in step 8.

9.Coloration: add 50 µL of substrate A, 50 µL of substrate B into each well. Mix gently by shaking, and incubate at 25 ℃for 15 min at dark.

11.Determination: add 50 µL stop solution into each well. Mix gently by shaking. then, Set the avelength of the microplate reader at 450 nm to determine the OD value of every well (in 5 min).

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 Malachite green concentration in the sample.

(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 Ⅰis 0.31, and that of the sample Ⅱis 0.82, the OD value of standard solutions is: 1.510 for 0 ppb, 1.320 for 0.025 ppb, 1.030 for 0.075 ppb, 0.660 for 0.225 ppb, 0.389 for 0.675 ppb ,0.198 for 2.025 ppb, accordingly the concentration range of the sample Ⅰis 0.675 to 2.025 ppb, and that of the sample Ⅱis 0.075 to 0.225 ppb.

(2) Quantitative determination

The mean values of the absorbance values 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

$$\text{Percentage of absorbance value} = \frac{B}{} \times 100\%$$

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$\frac{B}{B0}$

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 solutions and the semilogarithm values of the Clenbuterol standard solutions (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 Malachite green 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)

10. 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) 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 of the 0 standard solution (0 ppb) of less than 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.

11. Storage and expiry date

Storage: store at 2-8 ℃, not frozen.

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

Tips:

If the vacuum- package of Micro-well strips leaks,it won't influence the experiment results, feel at ease using it

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Green Earth depends on everyone' efforts

"Build of green Earth needs the cooperation of you and me"

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