VERSION 1.01

MALACHITE GREEN ELISA KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Malachite Green ELISA Test Kit

Catalogue Number. IP100022

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 content of and the content of Malachite green residues is subsequently obtained.

Technical specifications

Sensitivity: 0.05 ppb
Incubator temperature: 25℃
Incubator time: 30min～30min～15min
Detection limit: Aquatic products e.g. Shrimp, Fish 0.5 ppb, Water 0.1 ppb
Recovery rate: Shrimp, Fish, water 80%--110%
Cross-reaction rate: Malachite green 100%, Leucomalachite green 0.1%, Gentian violet 95%,, Leucocrystal violet 0.1%
Precision: CV ≤ 12%

Components

1	Micro-well strips	12 strips with 8 removable wells each	
2	10× standard solution (1mL each)	0 ppb, 0.5 ppb, 1.5 ppb, 4.5 ppb, 13.5 ppb, 40.5 ppb	
3	Enzyme conjugate	12ml	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
9	Oxidant	3ml	black cap
10	4× concentrated redissolving solution	50ml	transparent cap

Materials required but not provided

Equipments: microplate reader, homogenizer, nitrogen-drying device, vortex, oscillator, centrifuge, measuring pipets, balance (a sensibility reciprocal of 0.01 g), Incubator.
Micropipettors: single-channel 20-200 µL and 100-1000 µL, and multi-channel 30-300 µL.
Reagents: Acetonitrile (CH_3CN), Methylene chloride, N-hexane, Sodium sulfate (Na_2SO_4).

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 extracting solution:
Acetonitrile (CH_3CN) -Methylene chloride mixing solution: $V_{acetonitrile}$-$V_{methylene\ chloride}$ = 4 : 1, take 40 Acetonitrile (CH_3CN), then add 10ml Methylene chloride, mix evenly.
2) The 4× concentrated redissolving solution is diluted with deionized water at 1:3 (1 part 4× concentrated redissolving solution + 3 parts deionized water), or prepare as quantity needed.

Samples preparation

a) Samples preparation of Water

1) Take 50ul clear water sample to detect directly (if it is turbid water, must be filtered or centrifuge at 4000r/min for 10 min until get clear water). The unused water sample should store in frozen.
Fold of dilution of the sample: 1

b) Samples preparation of Aquatic products

1) Weigh 2±0.05g homogenized tissue sample into 50 ml centrifuge tube. Add 6mL Sample extracting solution,2g Sodium sulfate (Na_2SO_4), shake for 5 min, centrifuge at above 4000r/min at room temperature (25 ℃)for 10 min.
2) Take 3 mL supernatant, add 20ul Oxidant, shake evenly, blow to dryness by nitrogen or air at 56 ℃.
3) Add 1ml N-hexane and 1ml diluted sample redissolving solution, shake evenly for 1min, centrifuge at above 4000r/minfor 5 min.
4) Take 50 µL for analysis.
Fold of dilution of the sample: 1
(*Note: By this method, the result is total amount of Malachite Green, Leucomalachite green, Gentian violet and Leucocrystal violet.*)

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 kit to the room temperature (20-25 ℃) for at least 30 min, note that each reagent must be Shaken evenly before use.
2. Take the required micro-well strips and plate frames. Re-sealed the unused microplate, stored at 2- 8 ℃.
3. Dilute washing buffer: Dilute the 40 ml concentrated washing buffer (20x) with deionized water at 1:19 [1 part concentrated washing buffer (20x) + 19 parts deionized water], or prepare as quantity needed.

Prepare standard solution: dilute certain quantity 10X concentrated standard solution with diluted re-dissolving solution at 10 times, prepare for current use
See detail as following:

Standard 6	Take 50ul 40.5ppb standard, add 450ul diluted redissolving solution, mix evenly.	4.05ppb
Standard 5	Take 50ul 13.5ppb standard, add 450ul diluted redissolving solution, mix evenly	1.35ppb
Standard 4	Take 50ul 4.5ppb standard, add 450ul diluted redissolving solution, mix evenly.	0.45ppb
Standard 3	Take 50ul 1.5ppb standard, add 450ul diluted redissolving solution, mix evenly.	0.15ppb
Standard 2	Take50ul 0.5ppb standard, add 450ul diluted redissolving solution, mix evenly.	0.05ppb
Standard 1	Take 50ul 0ppb standard, add 450ul diluted redissolving solution, mix evenly	0ppb

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 into each well, then add antibody working solution, 50 µL/ well, mix gently by shaking the plate manually, seal the microplate with the cover membrane, and incubate at 25 ℃ for 30 min.
6. Pour the liquid out of microwell, wash the microplate with the washing buffer at 250 µL/well for 4-5 times,each time for 15-30 sec, flap to dry with absorbent paper
(if there are the bubbles after flapping, cut them with the clean tips).
7. Add 100 µL enzyme conjugate into each well, seal the microplate with the cover membrane, mix gently by shaking the plate manually, and incubate at 25 ℃ for 30 min, continue as described in step (6).
8. Coloration: add 50 µL substrate A then 50 µL substrate B into each well. Mix gently by shaking, and incubate at 25 ℃ for 15 min at dark.
9. Determination: add 50 µL stop solution into each well. Mix gently by shaking. Then, Set the wavelength of the microplate reader at 450 nm to determine the OD value of every well (Recommend to read the OD value at the dual-wavelength 450/630 nm 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 Malachite green concentration in the sample.

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 1.0, the OD value of standard solutions is: 2.243 for 0 ppb, 1.816 for 0.05 ppb, 1.415 for 0.15 ppb, 0.74 for 0.45 ppb, 0.313 for 1.35 ppb, 0.155 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.

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 solutions and the semilogarithm values of the Malachite green standard solutions (ng/mL) as Y- and X-axis respectively. Read the corresponding concentration of the samples from the standard curve by incorporating its absorption percentage into the standard curve.
The resulting value is subsequently multiplied by the corresponding dilution 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)

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. Put the unused microplate into an auto-sealing bag to re-seal it. The standard substance and the colorless color, former is light sensitive it 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

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