VERSION 1.01

MQCA ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

MQCA ELISA Test Kit

Catalogue Number. IP100049

Principle

This test kit is based on the competitive enzyme immunoassay for the detection of MQCA in the sample. The coupling antigens are pre-coated on the micro-well stripes. The MQCA in the sample and the coupling antigens pre-coated on the micro-well stripes compete for the anti-MQCA antibody. 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 MQCA in it. This value is compared to the standard curve and the MQCA concentration is subsequently obtained.

Technical specifications

Sensitivity: 0.5 ppb
Incubator temperature: 25℃
Incubator time: 30min～15min
Detection limit: Tissue 1 ppb
Cross-reaction rate: MQCA 100%, Olaquindox <0.1%
Recovery rate: Tissue 85%±10%

Components

1	Micro-well strips	12 strips with 8 removable wells each	
2	6× standard solution (1mL each)	0 ppb, 0.5 ppb, 1.5 ppb, 4.5 ppb, 13.5 ppb, 40.5 ppb	
3	Concentrated Enzyme conjugate	1ml	red cap
4	Antibody working solution	8ml	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	Redissolving solution	50ml	transparent cap

Materials required but not provided
Equipments: microplate reader (450 nm / 630 nm), homogenizer, oscillator, centrifuge, measuring pipets, nitrogen-drying device and balance (a sensibility reciprocal of 0.01 g), Incubator.
Micropipettors: single-channel 20 to 200 µL and 100 to 1000 µL and multi-channel 30～300 µl;
Reagents: Ethyl acetate, n-hexane, HCl, NaCl, CH3CN

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 checked to be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.

Samples preparation

Sample extract (Store at 2~8 ℃ for 1 month): Take 8.6ml concentrated HCl, add deionized water to 500ml, then add 10g NaCl to get the sample extract.

a) Livestock and poultry tissue (Chicken, duck, pork)

1. Take 2± 0.05 g of the homogenized sample into 50ml centrifuge tube;
2. Add 4 mL Sample extract, shake thoroughly for 1min;
3. Add 4ml Ethyl acetate and 2ml CH_3CN, shake properly for 10s; Centrifuge at 4000 r/min at room temperature (20 - 25 ℃) for 10 min (Note: This step only need shake for 10s, shake too long will lead to Emulsification, if Emulsification appeared, take a certain supernatant, then shake tube lightly for 5s, centrifuge again then can get enough supernatant);
4. Take 3 mL up-layer organic phase into a clear container, below to dry with Nitrogen or air at 56℃ water bath;
5. Add 1ml N-hexane, shake for 30s, then add 0.5ml Redissolving solution, shake and mix thoroughly for 30s; centrifuge at 4000 r/min at room temperature (20-25 ℃) for 5 min.
6. Remove the up-layer organic phase.Take 50 µL down-layer for analysis.

Fold of dilution of the sample: 0.5

ELISA procedures

Instructions

1) Bring all reagents and micro-well strips to 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) Take out all the necessary reagents and place at the room temperature (20-25 ℃) for at least 30min. Note that each reagent must be shaken to mix evenly before use;
2) Take the required micro-well strips and plate frames. Re-sealed the unused microplate, stored at 2-8 ℃;
3) Solution preparation: dilute 40 mL of the 20× concentrated washing buffer with deionized water at1 :19 (1 part 20× concentrated washing buffer + 19 parts deionized water), or prepare as quantity 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) Prepare mix solution of antibody working solution and Concentrated Enzyme conjugate: Mix antibody working solution and Concentrated Enzyme conjugate at 10:1 evenly (1000ul antibody working solution + 100ul Concentrated Enzyme conjugate, this mix solution should be used once prepared, it can not be stored, the package quantity has extra amount, prepare as proportion is OK.)
6) Add 20 µL the sample or standard solution to separate duplicate wells, then add 70 µL mix solution of antibody working solution and Concentrated Enzyme conjugate into each well. Mix by shaking gently, seal the microplate with the cover membrane, and incubate at 25℃ at dark for 30 min;

7) Wash the microplate with the washing buffer at 250 µL/well for four to five times; soak the well with the washing buffer for 15-30 sec, flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips);
8) Coloration: add 50 µL of the substrate A solution and 50 µL of the B solution into each well. Mix by shaking gently, and incubate at 25 ℃ for 15 min in the dark for coloration;
9) Determination: add 50 µL stop solution into each well. Mix by shaking gently. Set the wavelength of the microplate reader at 450 nm to determine the OD value. (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 content of MQCA.

Qualitative determination

The concentration range (ng/mL) can be obtained from the comparison 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, while those of the standard solutions are as the followings: 2.043 for 0 ppb, 1.716 for 0.5 ppb, 1.215 for 1.5 ppb, 0.74 for 4.5 ppb, 0.313 for 13.5 ppb and 0.155 for 40.5 ppb, accordingly the concentration range of the sample Ⅰ is 13.5 to 40.5ppb, and that of the sample Ⅱ is 1.5 to 4.5 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 MQCA 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, thus finally obtaining MQCA 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
3. Mix every reagent and reaction mixture evenly and wash the microplate thoroughly, otherwise there will be the undesirable reproducibility
4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin;

5. 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
6. 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
7. Discard the colouration solution with any color that indicates the degeneration of this solution. The detecting value of the 0 standard solution 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.