IMMUNOMART

VERSION 1.01

NITROFURAN (SEM) ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Nitrofuran (SEM) ELISA Test Kit

Catalogue Number. IP100004

Principle

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

Technical specifications

Sensitivity: 0.02ppb
Incubation Temperature: 25℃
Incubation Time: 30min～15min
Detection limit Tissue, egg, honey: 0.1ppb
Cross-reaction rate: AMOZ 100%, AHD<0.1%, AOZ<0.1%, SEM<0.1%
Recovery rate: Tissue 75±25%, Honey 70±20%, Egg 95±25%

Components

1	Micro-well strips	12 strips with 8 removable wells each	
2	6× standard solution (1 mL each)	0ppb, 0.02ppb, 0.06ppb, 0.18ppb, 0.54ppb, 1.62ppb	
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
9	2× concentrated redissolving solution	50ml	transparent cap
10	2-Nitrobenzaldehyde ($C_7H_5NO_3$)	10ml	black cap

Materials required but not provided

Equipments: microplate reader, printer, homogenizer, nitrogen-drying device, vortex, centrifuge, measuring pipets, balance (a reciprocal sensibility of 0.01 g), incubator, water bath;

Micropipettors: single-channel 20-200µL, 100-1000µL, and multi-channel 30～300µl;

Reagents: NaOH, ethyl acetate, n-Hexane, HCl (approx 36.5%), $K_2HPO_4 \cdot 3H_2O$

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) 0.1 M K_2HPO_4 : dissolve 11.4g $K_2HPO_4 \cdot 3H_2O$ in deionized water to 500mL.
2) 1 M HCl: dissolve 8.6mL HCI (approx 36.5%) in deionized water to 100mL.
3) 1 M NaOH: dissolve 4g NaOH in deionized water to 100mL.
4) the 2×concentrated redissolving solution is diluted with deionized water at 1:1(1mL concentrated redissolving solution + 1 mL deionized water), used for sample redissolving.

Samples preparation

a) Tissue, egg

1) Weigh 1± 0.05g of the homogenized sample, add 4mL of the distilled water, 0.5mL 1 M HCI and 100µL 2-Nitrobenzaldehyde ($C_7H_5NO_3$) to each tube, shake properly for 2min.
2) Incubate at 37 ℃ over night (approx 16 hours) or incubate at 56℃ by water bath(2 hours).
3) Add 5mL 0.1M K_2HPO_4, 0.4mL 1M NaOH and 6mL ethyl acetate to each tube, shake for 30s.
4) Centrifuge at above 4000r/min at room temperature (20-25 ℃) for 10min (if there is Emulsification or ethyl acetate layer is not enough for 3ml, incubate sample at 80℃ water bath for 10min and centrifuge repeatedly; or increase speed and extend time of centrifuge).
5) Transfer 3mL of the ethyl acetate layer into a new centrifugal tube and evaporate to dryness by nitrogen or air at 50℃.
6) Dissolve the dry residues in 2mL N-hexane, add 1mL of the diluted redissolving solution, mix properly for 30 seconds, centrifuge at above 4000 r/min at room temperature (20-25 ℃) for 10 min; Remove up-layer N-hexane phase (if there is Emulsification, after removing up-layer N-hexane phase, incubate sample at 70℃ water bath for 10-20min, centrifuge repeatedly).
7) Take 50 µL of the lower for analysis.
Fold of dilution of the sample: 2

b) Honey

1) Weigh 2± 0.05g of the homogenized sample (honey), add 4mL of the distilled water, 0.5mL 1 M HCI and 100 µL 2-Nitrobenzaldehyde ($C_7H_5NO_3$) to each tube, shake properly for 2min;.
2) Incubate at 37 ℃ over night (approx 16 hours) or incubate at 56℃ by water bath(2 hours).
3) Add 5mL 0.1M K_2HPO_4, 0.4mL 1M NaOH and 6mL ethyl acetate to each tube, shake for 30s.
4) Centrifuge at above 4000r/min at room temperature (20-25℃) for 10min (if there is Emulsification or ethyl acetate layer is not enough for 3ml, incubate sample at 80℃ water bath for 10min and centrifuge repeatedly; or increase speed and extend time of centrifuge).
5) Transfer 3mL of the ethyl acetate layer into a new centrifugal tube and evaporate to dryness by nitrogen or air at 50 ℃.
6) Dissolve the dry residues in 2mL N-hexane, add 1mL of the diluted redissolving solution, mix properly for 30 seconds, centrifuge at above 4000r/min at room temperature (20-25 ℃) for 10 min;

Remove up-layer N-hexane phase (if there is Emulsification, after removing up-layer N-hexane phase, incubate sample at 70℃ water bath for 10-20min, centrifuge repeatedly).
7) Take 50 μL of the lower for analysis.
Fold of dilution of the sample: 1

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 ELISA the procedures;
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 to mix evenly before use, put the required micro-well strips into plate frames. Re-sealed the unused microplate, store at 2-8 ℃, not frozen.
2. Solution preparation: dilute 40mL of the 20 × concentrated washing buffer with deionized water at 1:19 (1 part 20 × concentrated washing buffer + 19 parts deionized water). Or prepare as needed.
3. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate, record their positions.
4. Add 50μL of the sample or standard solution into separate duplicate wells; add 50ul enzyme conjugate then 50μL of the antibody working solution into each well, mix gently by shaking the plate manually. Seal the microplate with the cover membrane, and incubate at 25 ℃ for 30min.
5. Pour liquid out of microwell, flap to dry on absorbent paper, add 250μL/well of washing buffer to wash microplate for 15-30s, then take out and flap to dry with absorbent paper, repeat 4-5 times. (If there are the bubbles after flapping, cut them with the clean tips).
6. Coloration: add 50 μL of the substrate A and then 50 μL of the substrate B into each well. Mix gently by shaking the plate manually, then incubate at 25 ℃ for 15min at dark for coloration.
7. Determination: add 50μL of the stop solution into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450nm to determine the OD value of every well. (Recommend to read the OD value at the dual-wavelength 450/630nm within 5 minutes).

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 SEM.

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: 2.243 for 0ppb, 1.816 for 0.02ppb, 1.415 for 0.06ppb, 0.74 for 0.18ppb, 0.313 for 0.54ppb, 0.155 for 1.62ppb, accordingly the concentration range of the sample Ⅰ is 0.54 to 1.62ppb, and that of the sample Ⅱ is 0.06 to 0.18ppb.

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 (B_0) 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 SEM 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 SEM concentration in the sample.

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 lots to use.
6. Put the unused microplate into an auto-sealing bag to re-seal it. The standard solution 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 standard solution 1(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.