Nitrofuran (SEM) ELISA Test Kit

1. Principle

This test kit is based on the competitive enzyme immunoassay for the detection of semicarbazide (SEM) in chicken, fish, shrimp, milk, honey, whole egg. The coupling antigens are pre-coated on the micro-well stripes. The 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.

2. Technical specifications

Sensitivity: 0.1 ppb

Detection limit

Tissue, honey, egg ····· 0.2 ppb

Fish, shrimp (some interference in shrimp and fish) ····· 0.3 ppb

Recovery rate

Shrimp and fish ····· 85±10%

Honey ,chicken meat /liver,whole egg ····· 75±15%

Cross-reaction rate

SEM ····· 100%

AHD ····· < 0.1%

AMOZ ····· < 0.1%

AOZ ····· < 0.1%

3. Components

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

2） 6× standard solution (1 ml each): 0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb and 8.1 ppb

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

4） Antibody working solution (7 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

9） 2× concentrated redissolving solution (50 ml)..... transparent cap

10）2-Nitrobenzaldehyde($C_7H_5NO_3$) solution (10 ml).....white cap

4. Materials required but not provided

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

2) **Micropipettors:** single-channel 20 to 200 µl and 100 to 1000 µl, and multi-channel 250 µl.

3) **Reagents** NaOH, ethyl acetate, N-hexane, HCl(approx 36.5%), $K_2HPO_4 \cdot 3H_2O$, $K_2Fe(CN)_5NO \cdot 3H_2O$ and $ZnSO_4 \cdot 7H_2O$.

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 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) The 2× concentrated redissolving solution is mixed with deionized water at 1:1 (1 ml concentrated redissolving solution + 1 ml deionized water). used for sample redissolving.
2) *C solution* (for milk sample): dissolve 12.5 g $K_2Fe(CN)_5NO \cdot 3H_2O$ in deionized water to 100 ml.
3) *D solution* (for milk sample): dissolve 29.8 g $ZnSO_4 \cdot 7H_2O$ in deionized water to 100 ml.
4) 0.1 M K_2HPO_4 : dissolve 22.8 g $K_2HPO_4 \cdot 3H_2O$ in deionized water to 1 L.
5) 1 M HCl: dissolve 8.6 ml HCl (approx 36.5%) in water to 100 ml.
6) 1 M NaOH: dissolve 4 g NaOH in water to 100 ml.

5.1 Samples preparation

a) shrimp, fish and meat sample

- Homogenize the sample, continue as described in (1 to 7, **d**).

b) milk

- Put 5ml milk into centrifuge tube, add C and D solution, 250 µl each.
- Mix thoroughly, use vortex; centrifuge at above 4000r/min at 4-12 ℃ for 10 min with centrifuge of constant temperatures, if centrifuge of constant temperature is not available, chill sample temperature to approx 8 ℃, then centrifuge.
- Continue as described in (1 to 7, **d**).

C) honey

- Weigh 1 ± 0.05 g into centrifuge tube.
- Add 4 ml of the deionized water, then 0.5 ml 1 M HCl and 100 µl 2-Nitrobenzaldehyde solution are added, mix thoroughly.
- Continue as described in (2 to 7,**d**).

d) continue based on above steps

1. Weigh 1± 0.05 g of the homogenized sample (shrimp,fish,meat), 1.1 ml the supernatant of centrifugal milk(equivalent to 1 ml of milk sample), add 4 ml of the deionized water, 0.5 ml 1 M HCl and 100 µl 2-Nitrobenzaldehyde solution to each well, shake properly.
2. Incubate at 37 ℃ overnight (approx 16h).
3. Add 5 ml 0.1 M K_2HPO_4, 0.4 ml 1 M NaOH, 5 ml ethyl acetate to each tube, shake

vigorously for 30 s.

4. Centrifuge at above 4000 r/min at room temperature (20-25 ℃) for 10 min.
5. Transfer 2.5 ml ethyl acetate into a clean centrifuge tube and reduce to dryness by nitrogen or air at 50 ℃.
6. Dissolve the dry residues in 1 ml N-hexane, add 1 ml of the diluted redissolving solution, mix properly, centrifuge at above 4000 r/min at room temperature (20-25 ℃) for 10 min.
7. Take 50 µl of the lower layer for the analysis.

Fold of dilution of the sample: 2

e) whole egg

1. Weigh 2 g of the prepared egg sample, put into 50 ml centrifuge tube, add 4 ml of the deionized water, 0.5 ml 1 M HCl, 200 µl *C solution*, mix properly, then add 200 ul *D solution,* shake vigorously for 5 min, centrifuge at above 3000 r/min at room temperature (20-25 ℃) for 10 min.
2. Take all clear liquid (upper layer), add 200 µl 2-Nitrobenzaldehyde solution, heat in 50℃ waterbath for 2 h (shake for 1-2 min per 0.5 h), add 5 ml 0.1 M K_2HPO_4, 0.4 ml 1 M NaOH and 5 ml ethyl acetate, shake vigorously for 30 s.
3. Centrifuge at above 4000 r/min at room temperature (20-25 ℃) for 10 min.
4. Transfer 2.5 ml of the upper layer into a new centrifuge tube and evaporate to dryness by nitrogen or air at 50 ℃.
5. Dissolve the dry residue in 1 ml N-Hexane, add 2 ml of the diluted redissolving solution, mix properly ,shake for 10s, centrifuge at above 4000 r/min at room temperature (20-25 ℃) for 10 min(if an emulsion forms, place in waterbath at 60 ℃ for up to 5 min until separation occurs) , remove the organic phase (upper layer).
6. Take 50 µl aqueous phase (the lower) for analysis.

Fold of dilution of the sample: 2

6. ELISA procedures

1 Bring test kit to the room temperature (20 to 25 ℃) for at least 30min, note that each reagent must be shaken evenly before use, put the required micro-well strips into plate frames. Re-sealed the unused microplate, store at 2 to 8 ℃, not frozen.

2 Solution preparation: dilute 40 ml of the concentrated washing buffer (20×concentrated) with the distilled or deionized water to 800 ml (or just to the required volume) for use.

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 50 µl of the sample and 50 µl of standard solution to each well, then add 50 µl antibody to every well, mix gently by shaking the plate manually, seal the microplate with the cover membrane, and incubate at 37 ℃ for 30 min.

5 Pour the liquid, wash the microplate with the washing buffer at 250 µl/well for four to five times. Each time soak the well with the washing buffer for 10 s, flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips)

6 Add 100 µl enzyme conjugate into each well; and incubate at 37 ℃ for 30 min.Take out

microplate, continue as described in 5.

7 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 37 ℃ for 15 min at dark for coloration.

8 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. (Recommend to read the OD value at the dual-wavelength 450/630 nm within 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 content of SEM.

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 0.268, and that of the sample Ⅱ is 1.230, the OD value of standard solutions is: 1.671 for 0 ppb, 1.425 for 0.1 ppb, 1.103 for 0.3 ppb, 0.567 for 0.9 ppb, 0.205 for 2.7 ppb, 0.104 for 8.1 ppb, accordingly the concentration range of the sample Ⅰ is 0.9 to 2.7 ppb, and that of the sample Ⅱ is 0.10 to 0.30 ppb. (multiplied by the corresponding dilution fold)

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 (B_0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is,

$$\text{Percentage of absorbance value} = \frac{B}{B_0} \times 100\%$$

B—the average OD value of the sample or the standard solution

B_0—the average OD value of the 0ng/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.

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. Bring all reagents and micro-well strips to the room temperature (20 – 25 ℃) before use.

2. Return all reagents to 2 to 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.
5. The reagents and the samples should not return the room temperature (20 to 25 ℃) will lead to a lower standard OD value.
6. 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.
7. Mix evenly, otherwise there will be the undesirable reproducibility.
8. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.
9. 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.
10. 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.
11. 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.

9. Storage and expiry date

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

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

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