VERSION 1.01

NEOMYCIN ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Neomycin ELISA Test Kit

Catalogue Number. IP100005

Principle

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

Technical specifications

Sensitivity: 0.1ppb
Incubation Temperature: 25℃
Incubation Time: 30min～15min
Detection limit: Tissue (Chicken/liver, pork/liver, shrimp, fish): 4ppb; Honey: 4ppb; Milk, milk powder: 4ppb
Cross-reaction rate: Neomycin 100%, Streptomycin < 0.1%, Gentamicin < 0.1%, Dihydrostreptomycin < 0.1%, Kanamycin < 0.1%, Tobramycin < 0.1%, Sisomicin < 0.1%
Recovery rate: Tissue (chicken, pork, fish, shrimp), honey, milk, milk powder 90±30%, Liver (chicken or porcine) 70±17%

Components

1	Micro-well strips	12 strips with 8 removable wells each	
2	6× standard solution (1 mL each)	0ppb, 0.1ppb, 0.3ppb, 0.9ppb, 2.7ppb, 8.1ppb	
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

Materials required but not provided

Equipments: microplate reader, printer, homogenizer, vortex, centrifuge, measuring pipets, and balance(a sensibility reciprocal of 0.01g), incubator.
Micropipettors: single-channel 20-200μL, 100-1000μL, and multi-channel 30～300μl;
Reagents: Trichloroacetic acid(TCA), NaOH, deionized water.

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 checked to 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 (1mL concentrated redissolbing solution + 1mL deionized water).
2) 3% Trichloroacetic acid: dissolve 3g Trichloroacetic acid in the deionized water to 100mL.
3) 2% Trichloroacetic acid: dissolve 2g Trichloroacetic acid in the deionized water to 100mL
4) 2 M NaOH: dissolve 8g NaOH in the deionized water to 100mL

Samples preparation

a) Tissue(Chicken/liver, pork/liver, shrimp, fish) sample

1. Take 2±0.05g of the homogenized sample into 50mL centrifuge tube, add 8mL 3% Trichloroacetic acid, shake for 5min, centrifuge at above 4000 r/min at room temperature (20-25℃) for 10 min.
2. Take 2ml supernatant to another tube, adjust PH to 7.0-7.5 with 2M NaOH(about100ul), Dilute it with diluted redissolving solution at 1:4(100μL sample+400μL diluted redissolving solution), mix for 30s .
3. Take 50μL for analysis.

Fold of dilution of the sample: 20

b) Honey, milk, milk powder sample

1. Take 2±0.05g sample (2ml milk) into 50mL centrifuge tube, add 8mL 2% Trichloroacetic acid, shake for 5min, centrifuge at above 4000 r/min at room temperature (25℃) for 10min.
2. Take 2ml supernatant to another tube, adjust PH to 8.0 with 2M NaOH (about 100ul), Dilute it with diluted redissolving solution at 1:4(100μL sample+400μL diluted redissolving solution), mix for 30s .
3. Take 50 μL for analysis.

Fold of dilution of the sample: 20

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. Take out the kit from the refrigerated environment. Take out all the necessary reagents from the kit and place at the room temperature (20-25 ℃) for at least 30 min. 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, store at 2-8℃, not frozen.
3. Solution preparation: dilute 40mL of the 20× concentrated washing buffer with the deionized water at 1:19 (1 part 20× concentrated washing buffer + 19 parts deionized water) for use, 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. Add 50μL of the sample and standard solution to separate duplicate wells; Then 50μL enzyme conjugate and 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.
6. Pour the liquid, wash the microplate with the washing buffer at 250μL/well for 4-5 times. Each time 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).
7. Coloration: add 50μL of the substrate A and then 50μL of the substrate B into each well. Mix gently by shaking the plate manually, and incubate at 25 ℃ for 15 min at dark for coloration.
8. Determination: add 50μL of the stop solution into each well. Mix gently by shaking the plate manually. Set the wavelength of microplate reader at 450nm to determine the OD value. (recommend to read the OD value at the dual-wavelength 450/630nm 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 Neomycin.

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: 2.243 for 0ppb, 1.816 for 0.1ppb, 1.415 for 0.3ppb, 0.74 for 0.9ppb, 0.313 for 2.7ppb and 0.155 for 8.1ppb, accordingly the concentration range of the sample Ⅰ is 2.7 to 8.1ppb, and that of the sample Ⅱ is 0.3 to 0.9ppb.

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 Neomycin 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, thus finally obtaining the Neomycin 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.
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 standard solution 1 (0 ppb) of less than 0.5 indicates its degeneration.
8. The optimum reaction temperature is 25 ℃, If 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.