LSY-10005 Neomycin ELISA test kit

Neomycin ELISA Test Kit

Catalog no.: LSY-10005

1. Principle

This test kit is based on the indirect competitive enzyme immunoassay for the detection of Neomycin in the sample. 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.

2. Technical specifications

Sensitivity: 0.1ppb

Incubation Temperature: 25℃

Incubation Time: 30min—15min

Detection limit:

Tissue, milk, milk powder, feed: 4ppb

Duck: 8ppb

Honey: 7ppb

Egg: 3ppb

Pig urine: 1ppb

Recovery rate:

Tissue: 95±25%

Chicken liver, Pig liver: 80±25%

Milk, milk powder, honey, egg, feed, pig urine: 90±30%

Cross-reaction rate

Neomycin: 100%

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3. 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	15ml	white cap
9	2× concentrated redissolving solution	50ml*2	transparent cap

4. Materials required but not provided

1) Equipment: microplate reader, printer, homogenizer, vortex, centrifuge, measuring pipets, and balance (a sensibility reciprocal of 0.01g), incubator.

2) Micropipette: single-channel 20-200μL, 100-1000μL, andmulti-channel30～300μL.

3) Reagents: Trichloroacetic acid (TCA), NaOH, HCl, deionized water.

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 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) 3% Trichloroacetic acid: dissolve 3gTrichloroacetic acid in the deionized water to100mL.

2) 1 M NaOH solution: dissolve4gNaOHin the deionized water to100mL

3) 1 M HCl solution: take 8.6mlHCl, add deionized water to100mL

4) Sample dilution buffer: the 2×concentrated redissolving solution is mixed with deionized water at 1:1 (1 part of concentrated redissolving solution + 1 part of deionized water).

5) Sample extracting buffer: dilute Sample dilution buffer with deionized water at 1:9 (or directly dilute the2×concentrated redissolving solution with deionized water at 1:19).

5.1 Tissue (Chicken, pork, duck, shrimp, fish), honey, egg sample

1. Take 2±0.05g of the homogenized sample into 50mL centrifuge tube, add 8mL 3% Trichloroacetic acid, shake for4min, centrifuge at above4000 r/min at room temperature (20-25℃) for5 min.

2. Take 100μl up-layer clear liquid to another tube, add700μl of the Sample dilution buffer, shake thoroughly to mix it evenly.

3. Take50μL for analysis.

Fold of dilution of the sample: 40

5.2 Pig Liver sample

1. Take1±0.05g of the homogenized pig liver sample into 50mL centrifuge tube, add5mLof the Sample extracting buffer, shake for3min, put it at56℃water bath for 25min, vortex for 30s(or shake by hand for 30s),centrifuge at above4000 r/min at room temperature (20-25℃) for5 min.

2. Take 100μl up-layer clear liquid to another tube, add 300μl of the Sample dilution buffer, shake thoroughly to mix it evenly.

3. Take50μL for analysis.

Fold of dilution of the sample: 20

5.3 Feed sample

1. Take1±0.05g of the crushed feed sample into 50mL centrifuge tube, add9mLof the Sample extracting buffer and1ml 1M NaOH solution, shake for3min, centrifuge at above4000 r/min at room temperature (20-25℃) for5 min.

2. Take 100μl up-layer clear liquid to another tube, add 400μl of the Sample dilution buffer, shake thoroughly to mix it evenly.

3. Take50μL for analysis.

Fold of dilution of the sample: 50

5.4 Pig urine sample

1. Take1ml±0.05ml of the pig urine sample into 50mL centrifuge tube, add 5mLof the deionized water, shake for2min to mix it evenly.

2. Take 100µl of above liquid, add 300µl of the Sample dilution buffer, shake thoroughly to mix it evenly.

3. Take50µL for analysis.

Fold of dilution of the sample: 20

5.5 Milk sample

1. Take1ml±0.05ml of the milk sample into10mL centrifuge tube, add 50µl 1M HCl solution, shake for1min, centrifuge at above4000 r/min at room temperature (20-25℃) for5 min.

2. Take50µl up-layer clear liquid to another tube, add 950µl of the Sample dilution buffer, shake thoroughly to mix it evenly.

3. Take50µL for analysis.

Fold of dilution of the sample: 20

5.6 Milk powder sample

1. Take1g±0.05g of the milk powder sample into50mL centrifuge tube, add7ml deionized water, shake for3 min,centrifuge at above4000 r/min at room temperature (20-25℃) for5 min.

2. Take1ml up-layer liquid to another 10ml centrifuge tube, add50µl 1M HCl solution, shake for 1min,centrifuge at above4000 r/min at room temperature (20-25℃) for5 min.

3. Take 100µl up-layer clear liquid to another tube, add 900µl of the Sample dilution buffer, shake thoroughly to mix it evenly.

4. Take50µL for analysis.

Fold of dilution of the sample: 80

6. ELISA procedures

6.1 Instructions

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

2 Return all reagentsto2-8 ℃ immediately after use;

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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 procedure's temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.

6.2 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 15mL of the20× concentrated washing buffer with the deionized water at 1:19(1 part of 20× concentrated washing buffer + 19 parts of deionized water) for use, or prepare as quantity needed.

4. Numbering: number the micro-wells according to samples to samples; each sample to samples should be performed in duplicate, record their positions.

5. Add 50μL of the sample or standard solution to separate duplicate wells; Thenadd50μL enzyme conjugate, then add 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 at25 ℃ 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 scofflaw to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips).

7. Coloration: add50μL of the substrate A and then50μL of the substrate B into each well. Mix gently by shaking the plate manually, and incubate at 25 ℃ for 15minatdark for coloration.

8. Determination: add50μL of the stop solution into each well. Mix gently by shaking the plate manually. Set the wavelength the wavelength at 450nm to determine the OD value. (Recommend to read the OD value at the dual-wavelength 450/630nm within 5 min).

7. Result judgment

There are two methods to judge the results; the first one is through 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.

7.1 Qualitative determination

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The concentration range (ng/mL) can be obtained from the comparison deaveraged value of the sample with that of the standard deaveraged the OD value of the sample is0.3, and that of temple is 1.0, while those of the standard solutions are as the followings: 2.243 for 0ppb,1.816 for0.1ppb, 1.415for0.3ppb,0.74 for 0.9ppb,0.313 for 2.7ppb and 0.155 for 8.1ppb, accordingly the concentration range of thesampleIis2.7 to 8.1ppb, and that of the sample II is 0.3 to 0.9ppb.Multiplying by its corresponding dilution factor is the actual concentration of neomycin in the sample.

7.2 Quantitative determination

The mean values of the absorbance values obtained for the average OD value (B) of the sample and the standard solution divided by the OD value (B0) of solution divided(0 standard) solution divided by 100%, that is,

$$\text{Percentage of absorbance value} = \frac{B}{B0} \times 100\%$$

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

B0—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 semi logarithm 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.

8. 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 the2 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 colorless 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 insensitivity and the detecting OD values. Do not exchange the reagents from the kits of different lot numbers to use.

7. Discard the coloration 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 temperatureis25 ℃, If too high or too low temperatures will result in the changes in the detecting sensitivity and OD values.

9. Storage and expiry date

Storage: store at2-8 ℃, not frozen.

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

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