Technical Manual

Neo (Neomycin) ELISA Kit

- Catalogue Code: FSES0045
- Competitive ELISA Kit
- Research Use Only

Contents

1. Key features and Sample Types

Sensitivity:

0.5 ppb (ng/mL)

Assay Procedure:

25°C, 30 min ~15 min

Detection Limit:

Muscle, Egg - 50 ppb; Raw Milk - 20 ppb

Cross Reactivity:

Neomycin - 100%, Streptomycin, Kanamycin, Gentamicin - < 0.1%,

Sample Recovery rate:

90%±30%.

Storage:

2-8°C for 6 months.

Expiry:

See Kit Label

2. Storage

Store the kit at 2~8°C. Do not freeze any test kit components.
Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2-8°C.

3. Test Principle

This kit uses a Competitive-ELISA method. It can detect Neomycin (Neo) in samples, such as muscle, raw milk, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody working solution, standard and other supplementary reagents. The microtiter plate in this kit has been pre-coated with coupled antigen. During the reaction, Neo in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-Neo antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter plate well, and substrate reagent is added for color development. There is a negative correlation between the OD value of samples and the concentration of Neo. The concentration of Neo in the samples can be calculated by comparing the OD of the samples to the standard curve.

4. Kit Contents

Each kit contains reagents for 96 assays including:

No.	Component	96-WellKit
1	ELISA Microtiter Plate	96 wells
2	Standards	1 mL each (0ppb, 0.5ppb, 1.5ppb, 4.5ppb, 13.5ppb, 40.5ppb)
3	HRP Conjugate	7 mL
4	Antibody Working Solution	7 mL
5	Substrate Reagent A	6 mL
6	Substrate Reagent B	6 mL
7	Stop Solution	6 mL
8	20×Concentrated Wash Buffer	25 mL
9	5×Concentrated Sample Solution	30 mL
10	Plate Sealer	3 pieces
11	Sealed Bag	1 piece
12	Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Additional materials required:

Other materials required but not supplied

- **Instruments:** Microplate reader, Homogenizer, Vortex mixer, Centrifuge, Balance (sensitivity 0.01 g).
- **Micropipette:** Single channel (20-200 µL, 100-1000 µL), Multichannel (30-300 µL).
- **Reagents:** Trichloroacetic Acid ($C_2HCl_3O_2$), Concentrated H_2SO_4, NaCl

5. Experimental Preparation

Bring all reagents and samples to room temperature before use.

Open the microplate reader in advance, preheat the instrument, and set the testing parameters.

1. Sample pre-treatment Notice:

Experimental apparatus should be clean, and the pipette should be disposable to avoid cross- contamination during the experiment.

2. Solution preparation

Please prepare solution according to the number of samples. Don't use up all components in the kit at once!

Solution 1: 3% Trichloroacetic Acid Solution (*for livestock, egg sample*)

Dissolve 15 g of **Trichloroacetic Acid** to 500 mL with deionized water, mix fully.

Solution 2: 2 M H2SO4 Solution (*for raw milk sample*)

Dissolve 10.65 mL of **Concentrated H_2SO4** to 100 mL with deionized water (slowly add water), mix fully.

Solution 3: Wash Buffer

Dilute **20×Concentrated Wash Buffer** with deionized water.
(20×Concentrated Wash Buffer (V): Deionized water (V) = 1:19).

Solution 4: Tissue Solution (*for raw milk sample*)

Dilute 5 mL of **5×Concentrated Sample Solution** with 20 mL deionized water, add 0.25 g of **NaCl**, mix fully.

Solution 5: Raw Milk Solution (*for raw milk sample*)

Dilute 10 mL of **5×Concentrated Sample Solution** with 40 mL Wash Buffer, add 0.75g **NaCl**, mix fully.

3. Sample pre-treatment procedure

Targets may be distributed unevenly, resulting in no detection. To avoid this, ensure to take sufficient samples when sampling.

3.1 Pre-treatment of muscle (livestock), egg sample:

1. Weigh 1±0.01 g of homogenate sample in to 50 mL centrifuge tube.
2. Add 3 mL of **3% Trichloroacetic Acid Solution** (Solution 1)
3. Vortex for 1 min, centrifuge at 4000 r/min for 5 min at room temperature.
4. Take 100 µL of supernatant in to 400 µL of **Tissue Solution** (Solution 4). Vortex for 10s, mix fully.
5. Take 50 µL for analysis.
   Note: Sample dilution factor: 20, detection limit: 50 ppb.

3.2 Pre-treatment of raw milk sample:

1. Weigh 1 mL of fresh sample in to 4 mL centrifuge tube, add 50 µL of **2 M H2SO4 Solution** (Solution 2), and mix fully.
2. Centrifuge at 4000 r/min for 5min at room temperature.
3. Discard the upper fat layer. Take 50 µL of supernatant in to 950 µL of **Raw Milk Solution** (Solution 5). Vortex for 10s, mix fully

4. Take 50 µL for analysis.

Note: Sample dilution factor: 20, detection limit: 20 ppb.

6. Assay Procedure

Bring all reagents and samples to room temperature (25°C) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2~8°C.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. **Standard and Samples must be tested in duplicate**.
2. **Add Sample:** add 50 µL of **Standard or Sample** per well, add 50 µL of **HRP Conjugate**, then add 50 µL of **Antibody Working Solution** into each well. Cover the plate with plate sealer, vortex for 10s gently to mix thoroughly. Incubate at 25°C for 30 min away from direct sunlight.
3. **Wash:** uncover the sealer carefully, remove the liquid of each well. Immediately add 260 µL of **Wash Buffer** (Solution 3). Repeat wash procedure for 4 times, 30s intervals/time. Invert the plate and pat it against absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **Colour Development:** add 50 µL of **Substrate Reagent A** to each well, and then add 50µL of **Substrate Reagent B**. Gently vortex for 15s to mix thoroughly. Incubate at 25°C for 15 min away from direct sunlight. (The reaction time can be extended according to the actual colour change).
5. **Stop Reaction:** add 50 µL of **Stop Solution** to each well. Gently vortex for 10s to mix thoroughly
6. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader. This step should be finished in 5 min after stop reaction.

7. Data Analysis

1. Absorbance (%) = $A/A_0 \times 100\%$

A: Average absorbance of standard solution or sample A_0:
Average absorbance of 0 ppb Standard solution

2. Drawing and calculation of standard curve

Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add the average absorbance value to standard curve to get corresponding concentration. **If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.**

For this kit, it is more convenient to use professional analysis form for accurate and fast analysis on a large number of samples.

8. Notes

1. The overall OD value will be lower when reagents have not been brought to room temperature before use or room temperature is below 25°C.
2. If the wells turn dry during the washing procedure, it will lead to bad linear standard curve and poor repeatability. Operate the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the reproducibility of this ELISA kit.
4. ELISA Microtiter plate should be covered by plate sealer. Avoid the kit to strong light.
5. **Each reagent is optimized for use in the FSES0045. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other FSES0045 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue colour. When OD value of standard (concentration: 0) < 0.8 unit (A450nm < 0.8), it indicates the reagents are deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.
8. As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.
9. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
10. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
11. The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS, etc. can be used for quantitative confirmation.

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