Neomycin ELISA Kit

1. Background
Neomycin residue in the production of biological products may lead to abnormal reactions of human beings, thus strict MRLs have been established. This kit is a rapid test product for the determination of tetracycline residues which is sensitive, accurate and time-saving. It can considerably reduce the operation errors in the assay.

2. Test Principle
This kit is based on indirect-competitive ELISA technology. The microtiter wells are coated with coupling antigen. Neomycin residue in the sample competes with the antigen coated on the microtiter plate for the antibody. After the addition of enzyme conjugate, TMB substrate is used to show the color. Absorbance of the sample is negatively related to neomycin reside in it, after comparing with the Standard Curve, multiplied by the dilution factor, neomycin residue quantity in the sample can be calculated.

3. Applications
This kit can be used in quantitative and qualitative analysis of neomycin residue in biological samples.

4. Cross-reactions

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Cross-reaction</th>
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</thead>
<tbody>
<tr>
<td>Neomycin</td>
<td>100%</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>&lt;0.1%</td>
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<tr>
<td>Kanamycin</td>
<td>&lt;0.1%</td>
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<tr>
<td>Apramycin</td>
<td>&lt;0.1%</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>&lt;0.7%</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>&lt;0.1%</td>
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</tbody>
</table>

5. Materials Required
5.1 Equipments:
---Microtiter plate spectrophotometer (450nm/630nm)
---Polystyrene centrifuge tube: 2ml
---Micropipettes: 20μl-200μl, 100μl-1000μl
   250μl-multipipette

5.2 Reagents
---Deionized water

6. Kit Components
--- Microtiter plate with 96 wells coated with antigen
--- Standard solutions × 6 bottles: 1ml/bottle
   0ng/ml, 0.5ng/ml, 1.5ng/ml, 4.5ng/ml, 13.5ng/ml, 40.5ng/ml
--- Spiking standard solution: 1ml, 1μg/ml
--- Antibody solution (7ml) ………………… green cap
--- Enzyme conjugate (7ml) ………………… red cap
--- Substrate solution A (7ml) ……………… white cap
--- Substrate solution B (7ml) ……………… red cap
--- Stop solution (7ml) ………………… yellow cap
--- 20 × Concentrated wash solution (40ml) ………………… transparent cap
--- 2 × sample diluent (50ml) ……………… blue cap

7. Reagents Preparation

Solution 1: Wash solution
Dilute the 20× concentrated wash solution with deionized water in the volume ratio of 1:19 (e.g. 10ml of 20× concentrated wash solution + 190ml of deionized water), which will be used for washing the plates. This solution can be stored at 4°C for 1 month.

8. Sample Preparations
---Dilute 2 × sample diluent (kit components) with deionized water in the volume ratio of 1:1 (e.g. 10ml of 2 × sample diluents + 10ml of deionized water).
---Dilute the samples with the diluted sample diluent to obtain a proper neomycin concentration in it (0.5-40.5ng/ml), take 50μl per well for assay.

8.1 Notice and precautions before operation:

   a. Please use one-off tips in the process of experiment, and change the tips when absorb different reagent.
   b. Make sure that all experimental tools are clean, otherwise it will affect the assay result.

9. Assay process
9.1 Notice before assay

   9.1.1 Make sure all reagents and microwells are all at room temperature (20-25°C).
   9.1.2 Return all the rest reagents to 2-8°C immediately after used.

9.1.3 Washing the microwells correctly is an important step in the process of assay; it is the vital factor to the
reproducibility of the ELISA analysis.

9.1.4 Avoid the light and cover the microwells during incubation.

9.2 Assay Steps

9.2.1 Take all reagents out at room temperature (20-25℃) for more than 30min, homogenize before use.

9.2.2 Get the microwells needed out and return the rest into the zip-lock bag at 2-8℃ immediately.

9.2.3 Number: Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.

9.2.4 Add standard solution/sample, enzyme conjugate and antibody: Add 50µl of standard solution (kit components) or prepared sample to corresponding wells. Add 50µl of enzyme conjugate (kit components), 50µl of antibody (kit components). Mix gently by shaking the plate manually and incubate for 30min at 25℃ with cover.

9.2.5 Wash: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250µl of diluted wash solution (solution 1) at interval of 10s for 4-5 times. Absorb the residual water with absorbent paper (the rest air bubble can be eliminated with unused tip).

9.2.6 Coloration: Add 50µl of solution A (kit components) and 50µl of solution B (kit components) to each well. Mix gently by shaking the plate manually and incubate for 15 min at 25℃ with cover.

9.2.7 Measure: Add 50µl of the stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450nm (It's suggested measure with dual-wavelength of 450/630nm. Read the result within 5min after addition of stop solution. We can also measure by sight without stop solution in short of the ELISA reader).

9.2.8 Measure: Add 50µl of the stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450nm (It's suggested measure with dual-wavelength of 450/630nm. Read the result within 5min after addition of stop solution. We can also measure by sight without stop solution in short of the ELISA reader).

10. Results

10.1 Percentage absorbance

The mean values of the absorbance values obtained from the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%.

\[ \text{Absorbance(\%)} = \frac{B}{B_0} \times 100\% \]

B —— absorbance of standards or samples

B0 —— absorbance of zero standard (0ng/ml)

10.2 Standard Curve

---To draw a standard curve: The absorbance value of standards as y-axis, semi-logarithmic of the concentration of the standards (ng/ml) as x-axis.

---The neomycin concentration of each sample (ng/ml), which can be read from the calibration curve, multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

11. Sensitivity, accuracy and precision

Linear range: 0.5-40.5ng/ml

Accuracy: 85±10%

Precision: CV of the ELISA kit all less than 10%.

12. Notice

12.1 The mean values of the absorbance values obtained for the standards and the samples will be reduced if the reagents and samples have not been regulated to room temperature (20-25℃).

12.2 Do not allow microwells to dry between steps to avoid unsuccessful reproducibility and operate the next step immediately after tap the microwells holder.

12.3. Shake each reagent gently before using.

12.4. Keep your skin away from the stop solution for it is the 2M H2SO4 solution.

12.5 Don’t use the kits out of date. Don’t exchange the reagents of different batches, for it will drop the sensitivity.

12.6 Keep the ELISA kits at 2-8℃, do not freeze. Seal rest microwell plates Avoid straight sunlight for the standard sample and the colorless chromogenic reagent are sensitive to light.

12.7 Substrate solution should be abandoned if it turns colors. The reagents may be turn bad if the absorbance value (450/630nm) of the zero standard is less than 0.5 (A450nm<0.5).

12.8 The coloration reaction needs 15 min after adding Solution A and Solution B. And you can prolong the incubation time if the color is too light to be determined. Never exceed 30min, on the contrary, shorten the incubation time properly.

12.9 The optimal reaction temperature is 25℃. Higher or lower temperature will lead to the changes of sensitivity and absorbance values.

13. Storage condition and storage period

Storage condition: 2-8℃.

Storage period: 12 months.