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User's Manual

High Sensitivity Kanamycin ELISA Test Kit

REF **DEIA-WZ048V**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

High Sensitivity Kanamycin ELISA Test Kit is a competitive enzyme immunoassay for the quantitative analysis of Kanamycin in biological samples (cell lysate, urine, serum, recombinant protein, and fermentation liquid, etc.).

General Description

Kanamycin 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 kanamycin residues which is sensitive, accurate and time-saving. It can considerably reduce the operation errors in the assay.

Principles of Testing

The method is based on a competitive colorimetric ELISA assay. The Kanamycin-BSA has been coated in the plate wells. During the analysis, sample and Kanamycin antibody (Antibody #1) and HRP-Conjugate (HRP-Conjugated Antibody #2) are added to the wells for incubation. If the Kanamycin residue is present in the sample, it will compete with the Kanamycin on the plate wells for the Kanamycin antibody. The secondary antibody, tagged with a peroxidase enzyme, targets the primary antibody that is complexed to the drug coated on the plate wells. The resulting color intensity, after addition of the HRP substrate (TMB), has an inverse relationship with the Kanamycin residue concentration in the sample.

Reagents And Materials Provided

1. Kanamycin-coated Plate: 1x96-well Plate
2. Kanamycin Standards:

Negative control (white CAP tube): 1 mL

0.2 ng/mL (yellow CAP tube): 1 mL

0.6 ng/mL (orange CAP tube): 1 mL

1.8 ng/mL (pink cap tube): 1 mL

5.4 ng/mL(purple cap tube): 1 mL

16.2 ng/mL (blue cap tube): 1 mL

1000 ng/mL(spiking, optional, red cap tube): 1 mL

3. Kanamycin Ab#1: 6.0 mL
4. HRP-Conjugated Ab#2: 6.0 mL
5. 10×Sample diluent: 15 mL
6. 20×Wash Solution: 28 mL

7. Stop Buffer: 12 mL
8. TMB Substrate: 12 mL
9. 10×Sample Ext. buffer (optional): 15 mL
10. Sample Balance Buffer(optional): 2 mL

* If you are not planning to use the kit for over 1 month, store Kanamycin Standard Stock, Kanamycin Antibody #1 and HRP-Conjugated Antibody #2 at -20°C or in a freezer.

Materials Required But Not Supplied

1. Microtiter plate reader (450 nm)
2. Tissue Mixer (e.g. Omni Tissue Master Homogenizer)
3. Vortex mixer (e.g. Genie Vortex mixer from VWR)
4. 10, 20, 100 and 1000 µL pipettes
5. Multi-channel pipette: 50-300 µL (Optional)

Storage

Store the kit at 2-8°C. The shelf life is 12 months when the kit is properly stored.

Specimen Collection And Preparation

Be sure samples are properly stored. In general, samples should be refrigerated at 2-4°C for no more than 1-2 days. Freeze samples to a minimum of -20°C if they need to be stored for a longer period. Frozen samples can be thawed at room temps (20–25°C/ 68 – 77°F) or in a refrigerator before use.

Preparation of 1×Sample diluent:

Mix 1 volume of 10×Sample diluent with 9 volumes of distilled water.

Preparation of 1×Sample Ext. buffer:

Mix 1 volume of 10×Sample Ext. buffer with 9 volumes of distilled water.

Cell Lysate

1. Take 100 µL of cell lysate, add 900 µL of 1×Sample diluent Buffer, mix well.
2. Centrifuge for 5 minutes at 4000 x g.
3. Take 50 µL of the supernatant per well for the assay.

Note: *Dilution factor:* 10.

Shrimp/Fish/ Meat/Liver/Kidney

1. To 1.0 g of the homogenized sample, add 4.0 mL of 1×Sample Ext. buffer ,
2. Vortex the sample for 1 minute with vortex mixer.
3. Centrifuge the sample for 5 minutes at 4,000 x g.
4. Carefully transfer 200 µL of the top layer to a new tube, add 1.375 mL of 1X Sample diluent and 25 µL of Sample Balance Buffer, vortex for 30 seconds.

5. Use 50 µL per well for the assay.

Note: *Dilution factor:* 40.

Urine/Serum

1. Take 50 µL of urine or serum sample, add 1.2 mL of 1X Sample diluent , mix well.
2. Centrifuge for 5 minutes at 4,000 x g.
3. Take 50 µL of the supernatant per well for the assay.

Note: *Dilution factor:* 25.

Milk

1. Add 100 µL of milk sample into a centrifuge tube, add 900 µL of 1X Sample diluent .
2. Vortex vigorously for 30 seconds.
3. Centrifuge at 4,000 x g for 10 minutes.
4. Use 50 µL of the supernatant sample for the assay.

Note: *Dilution Factor:* 10

Recombinant protein, Fermentation liquid

1. Centrifuge 1 mL sample at 4,000 x g for 5 minutes.
2. Get the supernatant 50 µL sample to 200 µL 1X Sample diluent.
3. Use 50 µL per well for the assay.

Note: *Dilution factor:* 5. If needed, the sample can be further diluted with 1X Sample diluent.

Reconstitution And Storage

IMPORTANT: All reagents should be brought up to room temperature before use (1–2 hours at 20–25°C/ 68–77°F). Solutions should be prepared just prior to ELISA test. All reagents should be mixed by gently inverting or swirling prior to use. Prepare volumes that are needed for the number of wells being run. Do not return the reagents to the original stock tubes/bottles. Using disposable reservoirs when handling reagents can minimize the risk of contamination and is recommended.

1. Preparation of 1X Wash Solution

Mix 1 volume of the 20X Wash Solution with 19 volumes of distilled water.

Assay Procedure

Label the individual strips that will be used and aliquot reagents as the following example:

Component	Volume per Reaction	24 Reactions
Kanamycin Antibody #1	50 µL	1.2 mL
HRP-Conjugated Ab #2	50 µL	1.2 mL
1X Wash Solution	1.0 mL	24 mL
Stop Buffer	100 µL	2.4 mL
TMB Substrate	100 µL	2.4 mL

1. Add 50 µL of each Kanamycin Standards in duplicate into different wells (Add standards to plate only in the order from low concentration to high concentration).

2. Add 50 µL of each sample in duplicate into different sample wells.
3. Add 50 µL of HRP-Conjugated Ab#2 to each well and 50 µL of Kanamycin Antibody #1 to each well. Mix well by gently rocking the plate manually for 30s.
4. Incubate the plate for 30 minutes at room temperature (20–25°C/ 68–77°F). *(Avoid direct sunlight and cold bench tops during the incubation. Covering the microtiter plate while incubating is recommended).*
5. Wash the plate 4 times with 250 µL of 1X Wash Solution. After the last wash, invert the plate and gently tap the plate dry on paper towels (Perform the next step immediately after plate washings. Do not allow the plate to air dry between working steps).
6. Add 100 µL of TMB substrate. Time the reaction immediately after adding the substrate. Mix the solution by gently rocking the plate manually for 30s while incubating *(Do not put any substrate back to the original container to avoid any potential contamination. Any substrate solution exhibiting coloration is indicative of deterioration and should be discarded. Covering the microtiter plate while incubating is recommended).*
7. After incubating for 15 minutes at room temperature (20–25°C/ 68–77°F), add 100 µL of Stop Buffer to stop the enzyme reaction.
8. Read the plate as soon as possible following the addition of Stop Buffer on a plate reader with 450 nm wavelength (Before reading, use a lint-free wipe on the bottom of the plate to ensure no moisture or fingerprints interfere with the readings).

Calculation

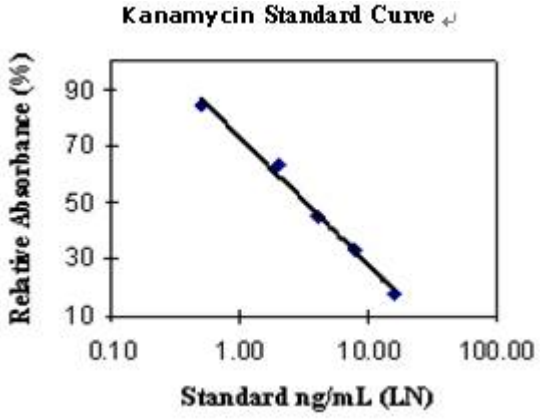
A standard curve can be constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ng/mL on a Logarithm curve.

Relative absorbance (%) = absorbance standard (or sample) x 100 / absorbance zero standard

When use computing software, recommends Log/Log standard curves.

Typical Standard Curve

The following figure is a typical kanamycin standard curve.



Sensitivity

Cell Lysate: 2ppb

Urine / Serum: 5ppb

Fermentation liquid: 1ppb

Recombinant protein: 1ppb

Specificity

Analytes	Cross-Reactivity (%)
Kanamycin	100
Streptomycin	< 0.1
Dihydrostreptomycin	< 0.1
Neomycin	< 0.1

Precautions

1. The standards contain Kanamycin. Handle with particular care.
2. Do not use the kit past the expiration date.
3. Do not intermix reagents from different kits or lots except for components with the same part No's within their expiration dates. ANTIBODIES AND PLATES ARE KIT-AND LOT-SPECIFIC.
4. Try to maintain a laboratory temperature of 20–25°C/(68°–77°F). Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation. Also, do not run assays in direct sunlight, as this may cause excessive heat and evaporation. Cold bench tops should be avoided by placing several layers of paper towel or some other insulation material under the assay plates during incubation.
5. Make sure you are using only distilled or deionized water since water quality is very important.
6. When pipetting samples or reagents into an empty microtiter plate, place the pipette tips in the lower corner of the well, making contact with the plastic.
7. Incubations of assay plates should be timed as precisely as possible. Be consistent when adding standards to the assay plate. Add your standards first and then your samples.
8. Add standards to plate only in the order from low concentration to high concentration as this will minimize the risk of compromising the standard curve.
9. Always refrigerate plates in sealed bags with a desiccant to maintain stability. Prevent condensation from forming on plates by allowing them equilibrate to room temperature (20 – 25°C/68 – 77°F) while in the packaging.