Version 25-04/25

User's Manual

High Sensitivity Streptomycin ELISA Test Kit

REF **DEIA-WZ020**

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 Streptomycin ELISA Test Kit is a competitive enzyme immunoassay for the quantitative analysis of streptomycin and dihydrostreptomycin in biological samples (serum and urine, etc.).

Principles of Testing

The method is based on a competitive colorimetric ELISA assay. The streptomycin has been coated in the plate wells. During the analysis, sample is added along with the HRP-Conjugated Antibody #2 and Streptomycin Antibody #1 . If the streptomycin residue is present in the sample, it will compete for the streptomycin antibody #1, thereby preventing the streptomycin from binding to the antibody attached to the well. The resulting color intensity, after addition of the HRP substrate (TMB), has an inverse relationship with the streptomycin residue concentration in the sample.

Reagents And Materials Provided

Streptomycin-coated Microtiter Plate 1 x 96-well plate (8 wells x 12 strips)

Streptomycin Standards:

Negative control (white cap tube): 1mL

0. 1 ng/mL (yellow cap tube): 1mL

0. 3 ng/mL (orange cap tube): 1mL

0. 9 ng/mL (pink cap tube): 1mL

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

8. 1 ng/mL (blue cap tube): 1mL

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

Streptomycin Antibody #1: 6mL

HRP-Conjugated Antibody #2: 6mL

10X Sample Extraction Buffer: 30 mL

10X Sample Dilution Buffer: 25 mL

20X Wash Solution: 30 mL

TMB Substrate: 12 mL

Stop Buffer: 12 mL

Materials Required But Not Supplied

1. Microtiter plate reader (450 nm)
2. Incubator
3. Tissue Mixer (e.g. Omni TissueMaster Homogenizer)

4. Vortex mixer (e.g. Gneie Vortex mixer from VWR)
5. 10, 20, 100 and 1000 uL pipettes
6. Multi-channel pipette: 50-300 uL (Optional)
7. n-hexane

Storage

2-8°C

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 temperature (20 – 25°C / 68 – 77°F) or in a refrigerator before use.

1. Preparation of 1X Sample Extraction Buffer

Mix 1 volume of 10X Sample Extraction Buffer with 9 volumes of distilled water.

2. Preparation of 1X Sample Dilution Buffer

Mix 1 volume of the 10X Sample Dilution Buffer with 9 volumes of distilled water.

3. Preparation of Phosphate Buffer

Weight 5.8g of Na2HPO4•12H2O and 0.592g of NaH2PO4•2H2O to 1000mL of distilled water.

Feed

1. To 1 g well homogenized representative feed sample in a 15 ml tube was added 5 mL of 1X Sample Extraction Buffer. Vortex for 3 minutes at maximum speed manually or 10 minutes using multiple-tube vortexor.
2. Centrifuge at 4,000 x g for 10 minutes at room temperature (20 –25°C / 68 –77°F).
3. Transfer 50 uL of the supernatant to a new tube, add 450 uL of Phosphate Buffer and mix well.
4. Use 50 uL per well for the assay.

Note: Dilution factor: 50. If needed, the sample obtained from step 3 can be further diluted with 1X Sample Dilution Buffer.

Honey

1. To 1 g well homogenized representative honey sample in a 15 ml tube was added 4 mL of 1X Sample Extraction Buffer. Vortex for 3 minutes at maximum speed manually or 10 minutes using multiple-tube vortexor.
2. Centrifuge at 4,000 x g for 10 minutes at room temperature (20 –25°C / 68 –77°F).
3. Transfer 100 uL of the supernatant to a new tube, add 300 uL of 1X Sample Dilution Buffer and mix well.
4. Use 50 ul per well for the assay.

Note: Dilution factor: 20. If needed, the sample obtained from step 3 can be further diluted with 1X Sample Dilution Buffer.

Meat/Liver/Kidney

1. To 2±0.05 g well homogenized representative tissue sample in a 15 ml tube was added 8 mL of 1X Sample Extraction Buffer. Vortex for 3 minutes at maximum speed manually or 10 minutes using multiple-tube vortexor.
2. Centrifuge at 4,000 x g for 10 minutes at room temperature (20 –25°C / 68 –77°F).
3. Transfer 1mL of the supernatant to a new tube, add 1mL of n-hexane and mix by vortexing at maximum speed for 2 minutes.
4. Centrifuge for 5 minutes at 4,000 x g at room temperature. Discard the upper hexane layer.
5. Transfer 50 uL of the sample to a new tube, add 450 uL of SampleDilution Buffer and mix well.
6. Use 50 uL per well for the assay.

Note: Dilution factor: 50. If needed, the sample obtained from step 6 can be further diluted with 1X Sample Dilution Buffer.

Milk

1. For fat-free milk, dilute the milk sample with 1X Sample Dilution Buffer (1:9) (e. g. 50 uL of milk + 450 uL of 1X Sample Dilution Buffer). Take 50 uLof the diluted sample per well for the assay.
2. For the regular milk with fat, centrifuge the milk sample at 4,000 x gfor 5 minutes, discard the upper fat layer. Dilute the sample with 1X Sample Dilution Buffer (1:9) (e. g. 50 uL of milk + 450 uL of 1X Sample Dilution Buffer). Take 50 uLof the diluted sample per well for the assay.

Note: Dilution factor: 10. If needed, the sample can be further diluted with 1X Sample Dilution Buffer.

Milk Powder

1. Weight 1 g of the sample in a 15 mL tube ,add 5 mL of distilled water. Vortex for 3 minutes at maximum speed manually or 10 minutes using multiple-tube vortexor.
2. Transfer 0.5 mL of the sample to a new tube was addde with 0.5 mL of 1X Sample Dilution Buffer.
3. Use 50 uL per well for the assay.

Note: Dilution factor: 10. If needed, the sample can be further diluted with 1X Sample Dilution Buffer.

Serum/Urine

1. Centrifuge 1 mL of the serum/urine sample at 4,000 x g for 5 minutes.
2. Take 100 uL of the supernatant and add 400 uL of 1X Sample Dilution Buffer. Vortex for 1 minute.
3. Use 50 uL per well for the assay.

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

Reagent Preparation

IMPORTANT: All reagents should be brought up to room temperature before use (1 – 2 hours at 20 – 25°C / 68 – 77°F); Make sure you read "Precautions" section. 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

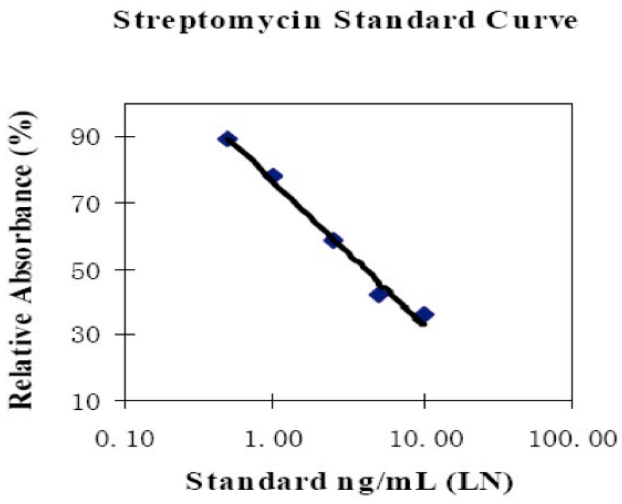
1. Add 50uL of each Streptomycin Standards in duplicate into different wells (Add standards to plate only in the order from low concentration to high concentration).
2. Add 50 uL of each sample in duplicate into different sample wells.
3. Add 50 uL of HRP-Conjugated Antibody #2 and 50uL Antibody #1 to each well , mix well by gently rocking the plate manually for 1 minute.
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 5 times with 250 uL 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 uL of TMB substrate. Time the reaction immediately after adding the substrate. Mix the solution by gently rocking the plate manually for 1 minute 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 uL 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 logarithmic curve.

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

Typical Standard Curve



Sensitivity

Serum/Urine: 0.5ppb

Specificity

Streptomycin ----------100

Precautions

1. The standards contain streptomycin. 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.