Version 20-04/24

User's Manual

Natamycin ELISA Kit

REF **DEIANJ13**

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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

Natamycin ELISA Test Kit enables government agencies, food manufacturers and quality assurance organizations to detect Natamycin to as low as 0.1 ug/g level in various sample types and to satisfy customer concerns about food safety.

The unique features of the kit:

The extraction of Natamycin from meat/tissue with a high recovery rate.

A quick ELISA assay (less than 1 hours regardless of number of samples).

High reproducibility Procedure Overview.

Principles of Testing

The method is based on a competitive colorimetric ELISA assay. The drug of interest has been coated in the plate wells. During the analysis, sample and HRP Conjugated Antibody are added along with the primary antibody specific for the target drug. If the target is present in the sample, it will compete for the antibody, thereby preventing the antibody from binding to the drug attached to the well. At the same time, the second 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 substrate, has an inverse relationship with the target concentration in the sample.

Reagents And Materials Provided

Natamycin ELISA Test Kit has the capacity for 96 determinations or testing of 42 samples in duplicate (assuming 12 wells for standards). Return any unused microwells to the foil bag and reseal them with the desiccant provided in the original package.

1. Natamycin Coated Plate, 1 x 96-well Plate (8 wells x 12 strips)
2. Natamycin Standards:(each of 1mL)

Negative control (white cap tube)

0.1 ug/mL (yellow cap tube)

0.25 ug/mL (orange cap tube)

0.5 ug/mL (pink cap tube)

1.0 ug/mL (purple cap tube)

5.0 ug/mL (blue cap tube)

100ug/mL (spiking, optional, red cap tube)

3. 10 X Natamycin Antibody 1#, 0.8 mL
4. Antibody Solution, 10 mL
5. HRP Conjugate Antibody 2#, 6.0 mL
6. 20X Wash Solution, 30 mL

7. Stop Buffer, 12 mL
8. 10 X Sample Extraction Buffer, 30 mL
9. TMB Substrate, 12 mL

Note: If you are not planning to use the kit for over 1 month, store Natamycin Standards, Natamycin Antibody and HRP-Conjugate at -20°C or in a freezer.

Materials Required But Not Supplied

1. Microtiter plate reader (450 nm)
2. Incubator
3. Rotary evaporator or nitrogen gas
4. Centrifuge
5. Vortex mixer (e.g. Genie Vortex mixer from VWR)
6. 10, 20, 100 and 1000uL pipette
7. Multi-channel pipette: 50-300uL (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 1X Natamycin Sample Extraction Buffer

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

Fresh Milk

1. For fat-free milk, take 50 uL of the sample per well for the assay.
2. For the regular milk with fat, centrifuge the milk sample at 4,000 rpm for 5 minutes, discard the upper fat layer.Use 50uL of clear supernatant per well for the assay.

Note: Dilution factor:1.

Pure Milk

1. Take 0.1 mL of the sample to 0.9 mL of 1X Sample Extraction Buffer,vortex well.
2. Use 50 µL of clear supernatant per well for the assay.

Note: Dilution factor: 10.

Milk Powder/Infant Formula

1. Take 1 g of milk powder/Infant formula, add 5 mL of distilled water.Vortex at maximum speed in a multi-

vortexer for 5 minutes or vortex for 10 minutes manually

2. Centrifuge at 4,000 rpm for 5 minutes.
3. Transfer 100 µL of clear supernatant to another tube, dilute with 300 µL of 1X Sample Extraction Buffer.
4. Vortex for 15 seconds.
5. Use 50 uL of the sample per well for the assay.

Note: Dilution factor: 20.

Meat/Tissue /Fish/Shrimp

1. Weigh 1 g of the sample.
2. Add 3 mL of Acetonitrile.Vortex at maximum speed in a multi-vortexer for 5 minutes or vortex for 10 minutes manually.
3. Centrifuge at 4,000 rpm for 5 minutes.
4. Transfer 1 mL of clear supernatant to another tube, Use a rotary evaporator to dry the sample in a 60-70°C water bath under reduced pressure. Alternatively,the sample can be dried by blowing nitrogen gas in a 60-70°C water bath.
5. Dissolve the dried residue in 2 mL of n-hexane (or n-heptane).
6. Add 0.5 mL of 1X Sample Extraction Buffer, vortex the sample for 2 minutes.
7. Centrifuge the sample at 4,000 x g for 5 minutes at room temperature (20-25°C / 68-77°F).
8. Use 50 uLof the lower aqueous layer per well for the assay.

Note: Dilution factor: 2.

Egg

1. Weigh out 1.0 g of egg sample .
2. Add 4 mL of 1 X Sample Extraction Buffer to the sample,Vortex at maximum speed in a multi-vortexer for 5 minutes or vortex for 10 minutes manually.
3. Centrifuge at 4000rpm for 10 minutes.
4. Transfer 200 uL of clear supernatant to another tube, dilute with 200 µL of 1X Sample Extraction Buffer.
5. Vortex for 15 seconds.
6. Use 50 uL of the sample per well for the assay.

Note: Dilution factor: 10.

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 only the volumes that are needed for the number of wells being run. Do not return the reagents to the original stock tubes/bottles. It is recommended that disposable reservoirs be used when handling reagents to minimize the risk of contamination.

Preparation of 1X Wash Solution

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

Preparation of 1X Natamycin Antibody1#

Mix 1 volume of the 10X Natamycin Antibody1# with 9 volumes of Antibody Solution

Assay Procedure

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

Component	Volume per Reaction	24 Reactions
1X Natamycin Antibody 1#	50 µL	1.2 mL
HRP Conjugate Antibody 2#	50 µL	1.2 mL
1X Wash Solution	2.0 mL	48mL
TMB Substrate	100 µL	2.4 mL
Stop Buffer	100 µL	2.4 mL

1. Add 50 uL of each Natamycin 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 Conjugate Antibody 2# to each well, then add 50 µL of 1X Natamycin 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).
5. Wash the plate 5 times with 300 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) * 100/absorbance zero standard

Use the mean relative absorbance values for each sample to determine the corresponding concentration of the tested drug in ng/mL from the standard curve.

Sensitivity

Fresh milk, 0.1ug/g

Egg/Pure milk, 1 ug/g

Milk powder/Infant Formula, 2 ug/g

Meat/Tissue/Fish/Shrimp,0.2 ug/g

Specificity

Natamycin, 100%

Ciprofloxacin, 42%

Danofloxacin, 32%

Enoxacin, 14%

Pipemidic acid, 11%

Benofloxacin, 3%

Enrofloxacin, 3%

Oxolin acid, 4%

Nalidixic acid, 2%

Precautions

1. The standards contain Natamycin. 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. Make sure that the Antibody and HRP Conjugate are mixed in correct volumes.
4. Try to maintain a laboratory temperature of 20°C–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 insulate 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 esiccant 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.