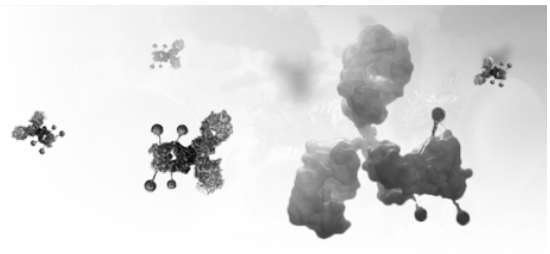


Nicarbazin ELISA Kit (DEIAPY1117N)

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

PRODUCT INFORMATION

Size 96T

General Description

The Nicarbazin ELISA Kit enables government agencies, food manufacturers and quality assurance organizations to detect nicarbazin in feed to satisfy customer concerns about food safety.

The unique features of the kit are:

1. The extraction of nicarbazin from samples can be finished in 10 - 30 minutes with a high recovery rate of 80 - 105%.
2. A quick ELISA assay (less than 1.5 hours regardless of number of samples).
3. High reproducibility.

Principles of Testing

The method is based on a competitive colorimetric ELISA assay. The nicarbazin antibody has been coated in the plate wells. During the analysis, sample is added along with the Nicarbazin-horseradish peroxidase conjugate. If the nicarbazin residue is present in the sample, it will compete for the nicarbazin antibody, thereby preventing the nicarbazin-HRP 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 nicarbazin residue concentration in the sample.

Reagents And Materials Provided

1. Nicarbazin Ab-Coated Plate 1x96-well Plate (8wells x12strips) 2-8°C
2. Nicarbazin Standards(2-8°C):

Negative control (white cap tube) 2.0 mL

1 ng/mL (yellow cap tube) 2.0 mL

2 ng/mL (orange cap tube) 2.0 mL

4 ng/mL (pink cap tube) 2.0 mL

8 ng/mL (purple cap tube) 2.0 mL

16 ng/mL (blue cap tube) 2.0 mL

16000ng/mL(spiking, optional, red cap tube) 2.0 mL

3. Nicarbazin-HRP Conjugate 6mL 2-8°C
4. 10X Sample Diluent ** 15 mL 2-8°C
5. 20X Wash Solution ** 30 mL 2-8°C
6. Stop Buffer ** 14 mL 2-8°C

7. TMB Substrate ** 12 mL 2-8°C

* If you are not planning to use the kit for over 3 months, store Nicarbazin-HRP Conjugate at -20°C or in a freezer.

Materials Required But Not Supplied

1. Microtiter plate reader (450 nm)
2. Tissue Mixer (e.g. Omni TissueMaster Homogenizer)
3. Vortex mixer (e.g. Genie Vortex mixer from VWR)
4. 10, 20, 100 and 1000 uL pipettes
5. Multi-channel pipette: 50-300 uL (Optional)
6. 10 mM PBS buffer: 0.24 g KH2PO4 + 1.44 g Na2HPO4 + 8 g NaCl, + 0.2 g KCl, adjust pH to 7.4 with NaOH, fill up to 1000 mL with distilled water

Storage

This 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. 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.

1. Preparation of 1x Sample Diluent:

Mix 1 volume of 10x Sample Diluent with 9 volumes of 10 mM PBS by inverting up and down for 10 times and incubate in the 37°C water bath for at least 30 minutes. Mix up and down again 10 times and make sure that the solution is homogenous.

Feed

1. Homogenize a reasonable amount of sample with a suitable mixer.
2. Weigh out 2 g of the homogenized sample and mix with 10 mL of acetonitrile.
3. Vortex for 1 minute at maximum speed.
4. Centrifuge for 10 minutes at 4,000 x g at room temperature (20 - 25°C / 68 - 77°F).
5. Transfer 20 µL of the supernatant to a 2 mL-tube and evaporate in mi-Vac Evaporator at 60°C or dry by blowing nitrogen gas
at 60°C.
6. Dissolve pellet in 1 mL of 1X Sample Diluent. Vortex for 1 minute to resuspend sample pellet.
7. Take 200 µL of above diluted sample, add 1800 µL of 1x Sample Diluent
8. Use 100 µL in the assay.

Note: Dilution factor: 50.

Meat, Tissue

1. Homogenize a reasonable amount of sample with a suitable mixer.
2. Weigh out 2 g of the homogenized sample and mix with 10 mL of acetonitrile,add 0.5g NaCl.
3. Vortex for 1 minute at maximum speed.
4. Centrifuge for 10 minutes at 4,000 x g at room temperature (20 - 25°C / 68 - 77°F).
5. Transfer 1 mL of the supernatant to a tube and evaporate in mi-Vac Evaporator at 60°C or dry by blowing nitrogen gas at 60°C.

6. Dissolve pellet in 1 mL of 1x Sample Diluent. Vortex for 1 minute to resuspend sample pellet.

7. Take 200 µL of above diluted sample, add 800 µL of 1X Sample Diluent

8. Use 100 µL in the assay.

Note: Dilution factor: 1.

Reagent Preparation

IMPORTANT: All reagents should be brought up to room temperature before use (1 - 2 hours at (2 0 - 2 5 ° C / 6 8 - 7 7 ° 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

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

Component	Volume per Reaction	24 Reactions
HRP Conjugate	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 100 µL of each Standards in duplicate into different wells (Add standards to plate only in the order from low concentration

to high concentration).

2. Add 100 µL of each sample in duplicate into different sample wells.

3. Add 50 µL of HRP Conjugate and mix well by gently rocking the plate manually for 1 minute.

4. Incubate the plate for 1 hour at room temperature (20 - 25°C / 68 - 77°F) in the dark.

5. Wash the plate 3 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 1 minute while incubating. Incubate for 20 minutes at room temperature (20 - 25°C / 68 - 77°F) in the dark. (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 incubation, 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).

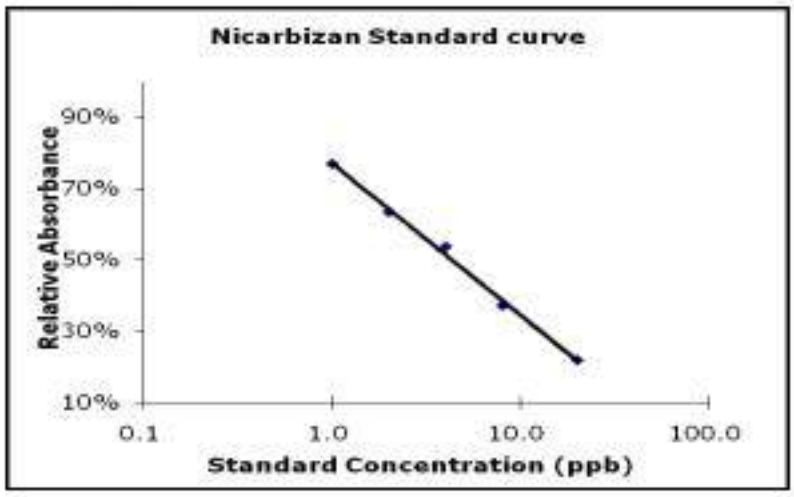
Calculation

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 logarithmic curve.

Relative absorbance (%) = Absorbance standard (or sample) x 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. A special program with Excel functionality.

Typical Standard Curve

The following figure is a typical chloramphenicol standard curve.



Detection Limit

Feed: 50 ng/g or ppb

Meat,tissue: 25 ng/g or ppb

Sensitivity

Analytes Cross-Reactivity (%)

Nicarbazin 100.0

Precautions

1. The standards contain Nicarbazin. 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. Nicarbazin-HRP CONJUGATES 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. making contact with the plastic.
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.