Intended Use

The Beacon Nifursol Plate Kit is an immunoassay for the detection of Nifursol and its metabolite, 3,5-Dinitrosalicylhydrazide (DNSH) residues in shrimp and fish tissues. This product is intended for research use only.

Principles

Nifursol HRP Enzyme Conjugate is pipetted into the red tabbed mixing wells followed by Calibrators and Sample Extract(s). The reagents are mixed and transferred to the test wells to initiate the reaction. Nifursol and DNSH in the calibrator/sample and Nifursol HRP Enzyme Conjugate compete for binding sites to the polyclonal Nifursol antibody immobilized on the test well surface. Following the incubation, the wells are washed to remove any unbound Nifursol, DNSH, and Nifursol HRP Enzyme Conjugate. After washing, a colorless substrate is added to the wells and any bound enzyme conjugate will convert the substrate to a blue color. Following an incubation, the reaction is stopped with the addition of Stop Solution and the amount of color in each well is measured. The color of the unknown sample is compared to the color of the calibrators and the Nifursol and DNSH concentration of the sample is derived. The color intensity is inversely proportional to the concentration of Nifursol and DNSH present in the sample.

Reagents and Materials Provided

1 Unit	Plate containing 12 antibody coated test strips of 8 wells each that are vacuum sealed in an aluminized pouch with a desiccant.
1 Unit	Plate containing 12 strips of 8 red tabbed mixing wells each that are packaged in a zip-loc bag.
6 X 2 mL	Vials of DNSH Calibrators (0, 0.04, 0.1, 0.25, 0.6 and 1.5 ppb).
6 Units	Vials of lyophilized Nifursol HRP Enzyme Conjugate (reconstitute prior to use).
1 X 50 mL	Bottle of HRP Enzyme Conjugate Diluent.
1 Unit	Packet of Wash Concentrate (dilute prior to use).
1 X 14 mL	Bottle of Substrate.
1 X 14 mL	Bottle of Stop Solution.
1 X 60 mL	Bottle of SX Buffer.

Reagents and Materials Required but Not Provided

- Pipette(s) with disposable tips capable of dispensing the required volume(s).
- Positive displacement pipette(s) with disposable tips capable of dispensing the required volume(s).
- Multichannel pipette(s) (8 channels) with disposable tips capable of dispensing the required volume(s).
- Laboratory grade water.
- Materials for wash solution preparation.
- Reagents and materials for Extraction Buffer preparation.
- Reagents and materials for 70% methanol preparation.
- Reagents and materials for the sample preparation.
- Personal protective equipment.
- Paper towels or equivalent absorbent material.
- Vortex.
- Timer.
- Microtiter plate or strip reader capable of reading at 450 nm.

Kit Handling Notes and Precautions

- Read the product brochure in its entirety prior to use.
- The kit, in its original packaging, can be used until the end of the month indicated on the box label.
- Do not use reagents after their expiration date.
- Store the lyophilized Nifursol HRP Enzyme Conjugate at -20°C when not in use. Store all other kit components at 2°C to 8°C (36°F to 46°F) when not in use.
- Kit components should be brought to room temperature, 20°C to 28°C (68°F to 82°F), prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- Do not freeze kit components (with the exception of the lyophilized Nifursol HRP Enzyme Conjugate) or expose them to temperatures greater than 37°C (99°F).
- Running Calibrators and Samples in duplicate will improve assay precision and accuracy.
- Precise transfer of samples and reagents by using a calibrated pipette that is capable of dispensing the required volume is critical to obtain proper assay results.
- If running more than two strips at once, the use of a multi-channel pipette is recommended when adding the Substrate and Stop Solution.
- All procedural steps should be completed without interruption. Ensure all reagents, materials and equipment are ready at the appropriate time.
- Each reagent is optimized for use in the Beacon Nifursol Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Nifursol Plate Kits with different lot numbers.
- Do not reuse test wells.
- Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- Damage to or obstruction of the optical surface may cause unsatisfactory results.

Specificity

The Beacon Nifursol Plate Kit is specific for Nifursol and DNSH residues with negligible reactivity with closely related compounds. The following table shows the relative values for the percent cross reactivity versus DNSH. All concentrations are in parts per billion (ppb).

Compound	% Cross-Reactivity
DNSH	100
Nifursol	151.7
AHD	<0.01
AMOZ	<0.01
AOZ	<0.01
SEM	<0.01

Limit of Detection (LOD) and Quantitation (LOQ)

The LOD and LOQ of this assay was determined through testing a statistically significant (n≥30) amount of negative control samples across different individuals, reagent lots, and days.

Sample Matrix	LOD (ppb)	LOQ (ppb)
Shrimp (Raw/Cooked, Wild/Farm Raised)	0.0286	0.0944
Fish (Farm Raised Fresh/Frozen Tilapia)	0.0312	0.106

Wash Solution Preparation

1. Measure 1 L of laboratory grade water and transfer to a clean container with a tight-fitting lid.
2. Transfer the contents of the Wash Concentrate packet to the container.
3. Gently swirl to mix.
4. Transfer the Wash Solution to a wash bottle to use in the assay.

HRP Enzyme Conjugate Preparation

1. Measure 5 mL of HRP Enzyme Conjugate Diluent and add to the lyophilized Nifursol HRP Enzyme Conjugate vial.
2. Vortex for 30 seconds and sit for 10 minutes to ensure the lyophilized powder is dissolved.
   Note: Unused reconstituted enzyme conjugate can be stored at 4°C. Long term storage (≥ 24 hours) at 4°C is not recommended.

Extraction Buffer Preparation (75% Methanol/SX buffer)

1. Measure 25 mL of SX buffer for each 100 mL being prepared and transfer to a clean glass container with a tight-fitting lid.
2. Measure 75 mL of methanol for each 100 mL being prepared and add to the container.
3. Cover and swirl to mix. Store tightly sealed to minimize evaporative loss.

70% Methanol Preparation (Dilution buffer as needed)

1. Measure 30 mL of laboratory grade water for each 100 mL being prepared and transfer to a clean glass container with a tight-fitting lid.
2. Measure 70 mL of methanol for each 100 mL being prepared and add to the container.
3. Cover and swirl to mix. Store tightly sealed to minimize evaporative loss.

Sample Preparation

Shrimp and Fish tissue: (5 mL / 1.0 g = Dilution Factor of 5X)

1. Finely homogenize the sample using a coffee grinder or blender.
2. Weigh 1 g of homogenized sample into a 15 mL disposable plastic conical tube.
3. Measure 5 mL of Extraction Buffer and add to the conical tube.
4. Vortex vigorously for 30 seconds. If the tissue clumps together, break up the clumps.
5. Incubate for a total of 30 minutes at room temperature. After 10 and 20 minutes have elapsed, vortex vigorously for 30 seconds each time. Let the mixture stand for the remaining 10 minutes.
6. Transfer 1 mL of the sample extract to a 1.5 – 2 mL microcentrifuge tube.
7. Centrifuge for 5 minutes at approximately 12,000 x g.
8. Transfer the clear supernatant to a new vial and use in the assay.

Assay Procedure

1. Allow kit components and the sample extract(s) to reach room temperature prior to running the test.
2. Place the appropriate number of red tabbed mixing wells and test wells into a holder. Be sure to reseal unused test wells in the zip-lock bag with the desiccant to limit exposure to moisture.
3. Dispense **100 µL of HRP Enzyme Conjugate** into each red tabbed mixing well.
4. Dispense **100 µL of Calibrators and Sample Extract(s)** into the appropriate red tabbed mixing well using a positive displacement pipette. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
5. Mix the contents of each well by gently pipetting up and down 10 times with a multichannel pipette and transfer **100 µL of the mixture** to the test wells.
6. Gently shake the wells for 30 seconds using a back-and-forth motion and incubate the test wells for **30 minutes** at room temperature. Discard the red tabbed mixing wells.
7. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with wash solution and then decant. Repeat this wash step four times for a total of five washes. Following the last wash, tap the inverted wells onto absorbent paper to remove excess wash solution.
8. Dispense **100 µL of Substrate** into each well.
9. Gently shake the wells for 30 seconds using a back-and-forth motion and incubate for **30 minutes** at room temperature.
10. Dispense **100 µL of Stop Solution** into each well as the same order of addition as the Substrate.
11. Carefully wipe the optical surface with a soft, lint-free wipe. Measure and record the absorbance (Optical Density; OD) of each well at 450 nm using a plate or strip reader within 10 minutes of stopping the assay. If the reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm.
12. Dispose of used test wells in an appropriate waste container.

Quality Control (QC) Criteria

- The correlation coefficient (R^2) of the calibration curve, analyzed using a 4-parameter logistic regression, must be ≥ 0.99.
- The average absorbance of the zero calibrator replicates must be ≥ 1.0.
- The average absorbance of calibrator replicates must have a coefficient of variation (%CV) < 15%.
- The average absorbance of sample replicates must have a coefficient of variation (%CV) < 20%.

Result Interpretation

It is preferred for quantitative results to be determined using commercially available software for ELISA evaluation using a 4-parameter curve fit. Alternatively, a semi-log curve fit can be used if 4-parameter software is not available. A spreadsheet that will perform the curve fit and sample concentration calculations is available upon request. Please contact Beacon for further details.

To ensure the validity of the results, please adhere to the following:

- Ensure QC criteria are met.
- The concentration of Nifursol in a sample is determined by comparing the average sample absorbance to the standard curve. This value must then be multiplied by the dilution factor used.
- In the event that the average absorbance of the sample is lower than the highest calibrator, further dilute the sample extract in 70% Methanol to fit into the standard curve and retest alongside the calibrators. Sample results must be multiplied by the total dilution factor used.

Technical Assistance
For questions regarding this kit or for additional information about Beacon products, contact us.

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Safety
The standard solutions in this test kit contain small amounts of DNSH. In addition, Stop Solution is 1N hydrochloric acid. Handle with care. To receive complete safety information, contact Beacon Analytical Systems, Inc., and request Safety Data Sheets.

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