Nitrofurazone (SEM) ELISA Kit

Enzyme-Linked Immunosorbent Assay for Detection of Nitrofurazone

Cat. No.: ELISA-SEM-001

96 TESTS

For *in vitro* use only

Please read this package insert completely before using this product

Background

Nitrofurazone belongs to the class of 5-nitrofuran antibiotics and has been widely and effectively used for the prevention and treatment of gastrointestinal infections caused by Escherichia coli and Salmonella spp. in cattle, pigs and poultry. It was also used as a growth promoter in food-producing animals. However, both WHO and the European Union (EU) are unable to assign a maximum residue limit for nitrofurazone because of the potential carcinogenic effects of its residues on human health. As a consequence, the administration of nitrofurazone to food-producing animals has been prohibited. Because of the rapid excretion of the nitrofurans and their instability in vitro and in vivo, it is impossible to monitor residues of the parent drug nitrofurazone directly. Instead, semicarbazide (SEM), the major metabolite of nitrofurazone, which is stable in tissue even after long-term storage, is selected for monitoring.

Intended use

The Biopanda SEM ELISA kit quantitatively detects the presence of SEM in meat, seafood, milk and honey samples.

Detection Limit - Seafood/Meat/Milk/Honey samples: 0.06 ppb

Recovery Rate - Seafood/Meat/Milk/Honey: 70-120%

Specificity - SEM: 100%
AHD:< 0.1%
AMOZ: < 0.1%
AOZ: < 0.1%

Principle

This test kit is based on the solid phase enzyme-linked immunosorbent assay (ELISA) for the detection of SEM. An unknown amount of SEM present in the sample and the fixed amount of SEM antigens pre-coated on the wells of microtiter plate/strips compete for the anti-SEM antibodies, which in turn is detected with Enzyme conjugate. After incubation the wells are washed and the bound enzyme is visualised by adding TMB solution. Any coloured product is measured at 450 nm after adding stop solution. The absorbance value of the developed colour is inversely proportional to the amount of the SEM in the sample. The quantity of SEM in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

Storage and stability

- The kit should be stored at 2–8°C. Do not freeze.
- Unused test wells should be sealed and stored at 2–8°C.
- This kit is valid until the expiration date printed on the label.

Kit components supplied

Item	Description
1	1 x Pre-coated microtiter plate (12 x 8 microwells)
2	1 x Enzyme conjugate solution (7 ml)
3	1 x SEM antibody solution (7 ml)
4	1 x Wash buffer concentrate (20×, 30 ml)
5	1 x Assay diluent concentrate (30×, 5 ml)
6	6 x SEM standards (0, 0.03, 0.1, 0.3, 0.9, 2.7 ppb, 1 ml each)
7	1 x High concentration of SEM standard (100 ppb, 1.0 ml)*
8	1 x Derivative reagent (10 ml)
9	1 x TMB solution (12 ml)
10	1 x Stop solution (12 ml)
11	1 x Microplate sealer
12	1 x Package Insert

**** This component is optional and only for the user to check the recovery rate of SEM.***

Materials/equipment required but not included with kit

- ELISA Microtiter plate reader equipped with 450 nm filter
- Multichannel micropipette and pipette tips
- 10-100, 100-1000 µl micropipette and micropipette tips
- Microplate washer or squeeze bottle
- Centrifuge
- Vortex
- Homogenizer
- Centrifugal tubes
- 10-50 ml tubes
- Deionized water
- Ethyl acetate
- N-hexane
- NaOH
- HCl
- $K_2HPO_4 \cdot 3H_2O$ (Potassium phosphate dibasic)
- $Na_2Fe(CN)_5NO \cdot 2H_2O$ (Sodium nitroprusside dihydrate)
- $ZnSO_4 \cdot 7H_2O$ (Zinc sulphate heptahydrate)

Precautions

- Please carefully read the instructions before use.
- Reagents should be brought to room temperature (RT, 20±5°C) prior to use.
- Do not use reagents after the expiration date. Do not use reagents from other kits with different Lot numbers.
- Avoid contact of skin and mucous membranes with reagents and sample extraction. If exposure should occur, immediately flush with water.
- Please wear protective gloves when using the kit. Consider all materials that are exposed to standards or samples to be contaminated.
- Use different tips when pipetting different reagents and samples.
- Keep the stop solution away from skin and eyes.

Preparation of working solutions

Wash buffer: dilute the Wash buffer concentrate (20×) 20-fold with deionized water (e.g. 10 ml Wash buffer concentrate with 190 ml H_2O).

Assay diluent: dilute the Assay diluent concentrate (30×) 30-fold with deionized water (e.g.1 ml Assay diluent concentrate with 29 ml H_2O).

1 M NaOH: Weigh out 4 g of sodium hydroxide and dissolve in deionized water for a final volume of 100 ml.

1 M HCl: Measure 8.6 ml of concentrated (approx. 37%) hydrochloric acid and add to 60 ml of deionized water. After mixing thoroughly, adjust the final volume to 100 ml with deionized water.

0.1 M K_2HPO_4: Weigh out 22.8 g of $K_2HPO_4 \cdot 3H_2O$ and dissolve in deionized water for a final volume of 1000 ml.

0.36 M $Na_2Fe(CN)_5NO \cdot 2H_2O$: Weigh out 10.7 g $Na_2Fe(CN)_5NO \cdot 2H_2O$ and dissolve in deionized water for a final volume of 100 ml.

1M $ZnSO_4 \cdot 7H_2O$: Weigh out 28.8 g of $ZnSO_4 \cdot 7H_2O$ and dissolve in deionized water for a final volume of 100 ml.

Note: Derivative reagent may be in solid form. Wash Buffer concentrate may also form crystals at low temperature. Ensure that the Derivative reagent and crystals completely re-dissolve before use (by placing into a 37°C incubator or water bath if necessary).

Sample Preparation

Meat/Seafood (dilution factor 2):

- Weigh 1 g of a homogenized sample into a 50 ml tube. Add 4 ml of deionized water, 1 ml 1 M HCl and 100 µl of derivative reagent. Vortex for 1 min and incubate at 56°C for 3 hours.
- Add 5 ml of 0.1 M K_2HPO_4, 900 µl 1 M NaOH, and 5 ml of ethyl acetate. Vortex for 5 min then centrifuge at 4000×*g* for 10 min at RT.

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- Collect 2.5 ml of supernatant and dry at 50°C with nitrogen gas.
- Add 1 ml N-hexane to dissolve the residue, then add 1 ml of diluted Assay Diluent. Vortex for 30 sec and centrifuge at 4000×*g* for 5 min at RT.
- Use 50 µl of the bottom layer solution as a sample in the assay.

Milk (dilution factor 2):

- Weigh 5 ml of a sample into a 50 ml tube. Add 250 µl of 0.36M $Na_2Fe(CN)_5NO•2H_2O$ solution. Vortex for 30 sec.
- Add 250 µl of 1M $ZnSO_4•7H_2O$ solution and vortex for 30 sec.
- Centrifuge at 4000×*g* for 10 min at RT.
- Collect 1.1 ml of supernatant and add 4 ml of deionized water, 1 ml of 1M HCl and 100 µl of derivative reagent.
- Vortex for 1 min then incubate at 56°C for 3 hours.
- Add 5 ml of 0.1 M K_2HPO_4, 900 µl 1 M NaOH, and 5 ml of ethyl acetate. Vortex for 5 min then centrifuge at 4000×*g* for 10 min at RT.
- Collect 2.5 ml of supernatant and dry at 50°C with nitrogen gas.
- Add 1 ml N-hexane to dissolve the residue, then add 1 ml of diluted Assay Diluent. Vortex for 30 sec and centrifuge at 4000×*g* for 5 min at RT.
- Use 50 µl of the bottom layer solution as a sample in the assay.

❖ ***The sample preparation method above is only a suggestion. The kit user can use their own methods to prepare samples.***

Test Procedure

1. Ensure all reagents are equilibrated to RT prior to use. Swirl all reagents gently before use.
2. Label each strip on its end tab to help identify them should they become detached from the plate frame during the assay.
3. To every well (except the two blank wells), add 50 µl of standard/sample to the wells in duplicate. Then add 50 µl of Enzyme conjugate solution to each well. Finally, add 50 µl of Antibody solution to each well.
4. To the two blank wells, add 100 µl of diluted Assay diluent and 50 µl of Enzyme conjugate (No standards/samples and antibody solution).
5. Shake gently to mix. Cover the strips with plate sealer. Incubate the plate for 30 minutes at 37°C.
6. After incubation, remove the plate sealer and wash the strips 5 times with diluted Wash buffer, ensuring every well is filled. When washing is completed, tap the strips firmly on absorbent tissue to remove residual Wash buffer.
7. Add 100 µl of the TMB solution to each well and incubate at 37°C for 10 minutes.
8. Stop the reaction by adding 100 µl of stop solution to each well in the same order as the TMB solution was added. Shake gently to mix.
9. Measure absorbance at 450 nm (with 630nm as a reference) within 10 minutes of stopping.

Test validity

For the test to be valid, the mean absorbance of the zero standard (0 ppb) must be over 1.0

Results calculation

The unknown SEM concentrations in the samples are determined from a standard curve. Calculate the mean absorbance value of the two blank wells and subtract that from the mean absorbance values of all the other wells.

Define the mean corrected absorbance value of the standards and samples as B. Define the mean corrected absorbance of the zero standard as B_0. The relative absorbance can therefore be calculated as:

$$Relative\ absorbance\ (\%) = \frac{B}{B_0} \times 100$$

Plot the relative absorbance of the standards against the standard concentration to obtain a standard curve. Using the relative absorbance value of a sample, the concentration can be found by interpolation. Remember to multiply by the dilution factor to obtain the true SEM concentration.

Interpolation can be performed by carrying out a 4-parameter logistic analysis, using a linear regression method, or point-to-point interpolation. Biopanda can provide an accompanying Excel spreadsheet calculator for this purpose.

Notes

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in this package insert and with adherence to good laboratory practice (GLP).
2. Factors that might affect the performance of the assay include proper instrument function/calibration, cleanliness of glassware, quality of distilled or deionised water, accuracy of reagent and sample pipetting, washing technique, incubation time and temperature.

A recommended plate layout is given as follows:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	T2	T2	T10	T10	T18	T18	T26	T26	T34	T34
B	S2	S2	T3	T3	T11	T11	T19	T19	T27	T27	T35	T35
C	S3	S3	T4	T4	T12	T12	T20	T20	T28	T28	T36	T36
D	S4	S4	T5	T5	T13	T13	T21	T21	T29	T29	T37	T37
E	S5	S5	T6	T6	T14	T14	T22	T22	T30	T30	T38	T38
F	S6	S6	T7	T7	T15	T15	T23	T23	T31	T31	T39	T39
G	B	B	T8	T8	T16	T16	T24	T24	T32	T32	T40	T40
H	T1	T1	T9	T9	T17	T17	T25	T25	T33	T33	T41	T41

'S' denotes the Standards in duplicate;
'B' denotes the Blank wells (see Step 4 of the Test Procedure);
'T' denotes the samples that are being tested in duplicate.

By following this recommended plate layout, the results from the microtiter plate reader can be copy & pasted directly into the accompanying spreadsheet calculator.

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