Sulfonamides ELISA Test Kit

Enzyme-Linked Immunosorbent Assay for Detection of Sulfonamides

Cat. No.: ELISA-SFN-001
96 TESTS
For *in vitro* use only
Please read this package insert completely before using this product

Background
Sulfonamides (SFN) are a group of synthetic antibacterial agents that contain the sulphonamide chemical group. SFN are widely used as feed additives and also in veterinary medicine for the treatment of various diseases. This can lead to SFN residues being found in animal source food leading to a variety of health hazards.

Intended use
The Biopanda Sulfonamides ELISA Test Kit is a competitive immunoassay to quantitatively detect the presence of SFN in honey, meat and seafood samples.

Detection Limit – Honey sample: 7.5 ppb
Meat sample: 7.5 ppb
Seafood sample: 7.5 ppb
Recovery Rate – Honey sample: 70-110%
Meat sample: 70-100%
Seafood sample: 100-120%
Specificity (Cross reactivity) – Sulfadiazine (SD): 100%
Sulfamonomethoxine (SMM): 3500%
Sulfisoxazole (SIZ): 18000%
Sulfamerazine (SM1): 380%
Sulfamethazine/Sulfadimidine (SM2): 1500%
Sulphamethoxydiazine (SMD) – 4500%
Sulfachloropyrazine (SCP): 4000%
Sulfadimethoxine (SDM): 4000%
Sulfaquinoxaline (SQX): 8000%
Sulfachloropyridazine: 18000%
Sulfadoxine: 1200%
Sulfamethizole: 150%
Sulfamethoxypyridazine (SMP): 900%
Sulfamethoxazole (SMX): 250%

Principle
This test kit is based on a competitive enzyme-linked immunosorbent assay (ELISA) for the detection of SFN. An unknown amount of SFN present in the sample and the fixed amount of SFN antigens pre-coated onto the wells of microtiter plate/strips compete for the anti-SFN antibodies, which in turn are 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 SFN in the sample. The quantity of SFN in the test sample can be calculated using the standard curve constructed from the standards, and corrected for the 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.

Item #	Description	Quantity
1	Pre-coated microtitre plate	12 x 8 wells
2	SFN antibody solution	1 x 5 ml
3	Enzyme conjugate	1 x 7 ml
4	Wash buffer (20× concentrate)	1 x 30 ml
5	Assay diluent (5× concentrate)	1 x 15 ml
6	SFN standards (0, 1.5, 4.5, 13.5, 40.5 ppb)	0.8 ml each
6	High concentrate of SFN standard (1 ppm)*	1 x 0.5 ml
7	TMB solution (ready to use)	1 x 12 ml
8	Stop solution (ready to use)	1 x 12 ml
9	Microplate sealer	1 piece
10	Package insert	1 copy

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

Materials/equipment required but not included with kit
- ELISA Microtiter plate reader equipped with 450/630 nm filters
- Single and multichannel micropipettes from 50 µl to 1.0 ml, with disposable pipette tips
- Plate shaking equipment
- Microplate washer or squeeze bottle
- Centrifuge
- Vortexer
- Centrifugal tubes
- Eppendorf tubes
- Deionised water (ddH_2O)
- Absorbent pads (tissue)
- Ethyl acetate*

****These materials may not be needed depending on the type of sample being tested***

Precautions
- Please carefully read the instructions before use.
- Reagents should be brought to room temperature (RT, 18-25°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. If exposure should occur, immediately flush with water.
- Please wear protective gloves when using the kit and consider that 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: Wash buffer concentrate (20×) must be diluted to working concentration and mixed prior to use (e.g. 10 ml wash buffer concentrate into 190 ml ddH_2O)
Assay Diluent: Assay diluent concentrate (5×) must be diluted to working concentration and mixed prior to use (e.g. 10 ml assay diluent concentrate with 40ml ddH_2O)

Note: Wash buffer concentrate may form crystals at low temperature. Ensure that the crystals completely re-dissolve before dilution (by placing into a 37°C incubator or water bath if necessary).

Sample Preparation

Honey (Dilution factor: 3)

- Weigh 1 g of honey into a 15 ml centrifugal tube.
- Add 2 ml ddH_2O and mix well (If honey is not fully dissolved, heat the honey at 37°C and mix well).
- Use this solution as a sample in the assay.

Meat / Seafood (Dilution factor: 6)

- Weigh 1 g of a homogenised sample into a 15 ml centrifugal tube.
- Add 5 ml of Ethyl acetate and mix for 2 minutes.
- Centrifuge the sample at 4000 × *g* for 10 minutes.
- Transfer 1 ml of the supernatant to an Eppendorf tube, and dry with nitrogen gas at 50°C.
- Add 1 ml of diluted assay diluent to dissolve the residue and use this solution as a sample in the assay.

**** Samples should be tested as soon as possible after preparation***

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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. Surplus wells should be placed back into the re-sealable foil pouch with desiccant and stored at 2-8°C.
3. To every well (except the two blank wells) add 70 µl of each standard or sample solution per well in duplicate, then add 50 µl of enzyme conjugate to each well, finally add 30 µl antibody solution to each well. **(Note: The order of addition is very important)**
4. To the two blank wells, add 100 µl of diluted assay diluent and 50 µl Enzyme conjugate (No standards/samples or antibody solution).
5. Cover the strips with the microplate sealer and shake gently to mix for 1 minute. Incubate the plate for 30 minutes at 37°C in the dark.
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 for 10 minutes at 37°C in the dark.
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 filter) within 10 minutes of stopping.

Test validity

For the test to be valid, the mean absorbance of the zero standard (S1, 0 ppb) must be over 1.5

Results calculation

The unknown SFN 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 SFN 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.

Thank you for purchasing Biopanda's Sulfonamides ELISA Test Kit. Please read this manual carefully before operating to ensure proper use.

A recommended plate layout is shown below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	T3	T3	T11	T11	T19	T19	T27	T27	T35	T35
B	S2	S2	T4	T4	T12	T12	T20	T20	T28	T28	T36	T36
C	S3	S3	T5	T5	T13	T13	T21	T21	T29	T29	T37	T37
D	S4	S4	T6	T6	T14	T14	T22	T22	T30	T30	T38	T38
E	S5	S5	T7	T7	T15	T15	T23	T23	T31	T31	T39	T39
F	B	B	T8	T8	T16	T16	T24	T24	T32	T32	T40	T40
G	T1	T1	T9	T9	T17	T17	T25	T25	T33	T33	T41	T41
H	T2	T2	T10	T10	T18	T18	T26	T26	T34	T34	T42	T42

'S' denotes the Standards in duplicate, and 'S1' denotes the Zero Standard;
'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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Biopanda Reagents

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