

AOZ (Nitrofurantoin) Lateral Flow Assay Kit

Catalog No: E-FS-C002

20T/50T/80T

Version Number:	V1.3
Replace version:	V1.2
Revision Date:	2025.04.17

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

This kit uses the principle of Immunochromatography assay for the qualitative detection. It can detect Nitrofurantoin (AOZ) in samples, such as fish and shrimp. After adding the sample solution into the sample well of detection card, AOZ in the sample solution combine with the gold-labelled antibody, so as to prevent the combining of gold-labelled antibody with AOZ conjugate on the cellulose membrane. When the concentration of AOZ in the sample solution is more than the detection limit, the detect line do not show color and the result is positive. When the concentration of AOZ in the sample solution is less than the detection limit, the detect line show color and the result is negative.

Technical indicator

Detection limit: Fish, Shrimp---0.5 ppb

Kits components

Item	Specifications
Detection Card (with disposable dropper and gold-labelled micro well)	50 T/kit
Reagent A	1 vial
Reagent B	1 vial
Extractant	1 vial
Purifying Agent	1 vial
Derivatization Reagent	1 vial
Reconstitution Buffer	1 vial
0.3M K ₂ HPO ₄	1 vial
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Other materials required but not supplied

Instruments: Homogenizer, Nitrogen Evaporators, Water bath, Oscillators, Centrifuge, Graduated pipette, Balance (sensitivity 0.01g), 1.5 mL Centrifuge tube, 50 mL Centrifuge tube.

High-precision transferpettor: Single channel (20-200 µL, 100-1000 µL).

Notes

1. FOR RESEARCH USE ONLY. Do not use product out of date or in a broken aluminum foil.
2. The detection card should be adjusted to room temperature after removed from the refrigerator before opening. The opening detection card should be used as soon as possible so as not to be invalid because of moisture.
3. Avoid of contacting the white membrane at the middle of the sample well.
4. The disposable dropper cannot be mixing to avoid the cross-contaminant.
5. The tested sample should be clear, no turbidity particle and no bacterial pollution, otherwise it is easy to result in abnormal phenomena such as obstruction, unobvious color, etc., which affect the judgment of the experiment result.
6. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
7. The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS, etc. can be used for quantitative confirmation.
8. **Each reagent is optimized for use in the E-FS-C002. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-C002 with different lot numbers.**

Storage and expiry date

Storage: Store at 2-30°C. With cool and dry environment.

Expiry date: expiration date is on the packing box.

Sample pretreatment

Restore all reagents and samples to room temperature before use.

1. Sample pretreatment Notice:

Experimental apparatus should be clean, and the disposable dropper should be disposable to avoid the experiment result be interfered by the contamination.

2. Reagent preparation

Solution 1: 0.1 M K_2HPO_4 Solution

Dilute the **0.3 M K_2HPO_4** with deionized water. (0.1 M K_2HPO_4 : Deionized water=1:2).

3. Sample pretreatment procedure:

3.1 Pretreatment of Fish, Shrimp sample:

- (1) Remove fat from sample, homogenize the sample with homogenizer.
- (2) Weigh 3 ± 0.05 g of homogenate sample into 15mL centrifuge tube, add 3 mL of deionized water, 0.3 mL of **Reagent A** and 0.2 mL of **Derivatization Reagent**, oscillate for 1 min.
- (3) Incubate for 10 minutes in water bath at 90 °C.
- (4) Add 2 mL of **0.1 M K₂HPO₄ Solution** (Solution 1), 0.2 mL of **Reagent B**, Shake vigorously for 30 seconds to mix well, Then add 4 mL of **Extractant**, oscillate for 1 min.
- (5) Centrifuge at 4000 r/min at room temperature for 3 min.
- (6) Take 2 mL of upper liquid to another tube, dry in nitrogen evaporators or water bath at 60 °C . (Please do it in a ventilated environment.)
- (7) Add 1 mL of **Purifying Agent** to the dried centrifuge tube, and first completely dissolve any residual substances on the tube wall by vortexing. Then, add 0.6 mL of **Reconstitution Buffer** and mix thoroughly for 30 seconds. Allow the mixture to stand for 1 minute until it clearly separates into layers.
- (8) Draw up 120µL of the lower layer solution after separation; this solution is the sample ready for testing.

Note: Detection limit: 0.5 ppb

Experiment procedure

1. Tear the aluminum foil bag of the detection card and take out the detection card, droppe and gold-labelled micro well, put it on a smooth, clean table (Before use, restore the card to be tested and the sample to be tested to room temperature).
2. Use a dropper to draw 120µL (approximately 4 drops) of the sample solution to be tested from the above sample. Gently pipette for 30 seconds to completely dissolve the red substance at the bottom of the gold-labelled micro well. Let it stand horizontally and wait for 2 minutes for the reaction. Then, draw the red solution from the gold-labelled micro well, gently pipette for 10 seconds, and add all of it to the sample well (S) of the test card. Start timing..
3. Incubate for 5 to 8 minutes and then judge the results immediately.

Judgment of result

1. **Negative:** The control line region (C) show color, the test line region (T) shows equal or darker than line C. It indicates the content of AOZ in the sample is lower than detection limit or the sample doesn't contain AOZ.
2. **Positive:** The control line region (C) show color, the test line region (T) shows no color or lighter color than line C. It indicates the content of AOZ in the sample is higher than detection limit.
3. **Invalid:** The control line region (C) shows no color. It indicates operation process is wrong or the test card is invalid.

