

AOZ (Nitrofurantoin) Lateral Flow Assay Kit

Catalog No: E-FS-C002

20T/50T/80T

Version Number:	V1.1
Replace version:	V1.0
Revision Date:	2024.3.11

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.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.elabscience.com

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 honey, muscle, liver, etc. 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: Honey, Muscle, Liver---0.5 ppb.

Kits components

Item	Specifications
Detection Card (with disposable dropper)	50 T/kit
Reconstitution Buffer	1 vial
Derivatization reagent	4 vials
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, Centrifuge, Graduated pipette, Balance (sensitivity 0.01g), Oscillators.

High-precision transferpettor: Single channel (20-200 μ L, 100-1000 μ L)

Reagents: Ethyl acetate, N-hexane, NaOH, Concentrated HCl, $K_2HPO_4 \cdot 3H_2O$

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 Do not use product out of date or in a broken aluminum foil.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 (25°C) 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: 0.5 M K_2HPO_4 Solution

Dissolve 11.4 g $K_2HPO_4 \cdot 3H_2O$ to 100 mL with deionized water

Solution 2: 1 M HCl Solution

Dilute 8.6 mL **Concentrated HCl** to 100 mL with deionized water

Solution 3: 1 M NaOH Solution

Dissolve 4 g **NaOH** to 100 mL with deionized water

3. Sample pretreatment procedure:

3.1 Pretreatment of honey, muscle, liver sample:

- (1) Remove fat from sample (except honey), homogenize the sample with homogenizer.
- (2) Weigh 2 ± 0.05 g of homogenate sample into centrifuge tube, add 4 mL of deionized water, 0.5 mL of **1 M HCl Solution** (Solution 2) and 600 μ L of **Derivatization reagent**, oscillate for 5 min.
- (3) Incubate for 30 minutes in water bath at 65 °C.
- (4) Add 1 mL of **0.5 M K₂HPO₄ Solution** (Solution 1), 0.4 mL of **1 M NaOH Solution** (Solution 3) and 5 mL of **Ethyl acetate**, oscillate for 5 min.
- (5) Centrifuge at 4000 r/min at room temperature for 5 min.
- (6) Take 2.5 mL of upper liquid to another tube, dry in nitrogen evaporators/water bath at 50-60 °C. (Please do it in a ventilated environment.)
- (7) Dissolve the residual with 1 mL **N-hexane**, add 0.5 mL of **Reconstitution Buffer** and oscillate for 30 s. Centrifuge at 4000 r/min at room temperature for 5 min.
- (8) Discard the upper n-hexane, take the lower liquid to analysis.

Note: Detection limit: 0.5 ppb

Experiment procedure

1. Tear the aluminum foil bag of the detection card and take out the detection card, and put it on a smooth, clean table.
2. Take the prepared clear sample with the matching disposable dropper, add 2-3 drops (about 60 μ L) of sample to the sample well (S) vertically and slowly (Avoid foaming).
3. Incubate for 8 to 10 minutes and then judge the results immediately.

Judgment of result

1. **Negative:** The control line region (C) and the test line region (T) both show a line in the observation well. It indicates the content of AOZ in the sample is lower than detection limit or the sample doesn't contain AOZ.
2. **Positive:** Only the control line region (C) show a line in the observation well. It indicates the content of AOZ in the sample is higher than detection limit.
3. **Invalid:** The control line region (C) does not show a line in the observation well. It indicates operation process is wrong or the test card is invalid.

