

Nitrofurans are a class of drugs typically used as antibiotics or antimicrobials. The defining structural component is a furan ring with a nitro group. This product utilizes the high affinity of monoclonal antibody against nitrofurans metabolites, which can easily identify its contamination in various samples.

## 1. Application

This kit can be used to qualitatively detect nitrofurans metabolites in shrimp, meat and egg.

## 2. Detection Limit (LOD) in various samples (ppb)

Analyte	T Lines	Detection Limit
Furaltadone metabolites AMOZ	T1	0.5 - 0.8
Nitrofurazone metabolites SEM	T2	0.4 - 0.5
Furazolidone metabolites AOZ	T3	0.4 - 0.5
Nitrofurantoin metabolites AHD	T4	0.4 - 0.5

## 3. Kit components

- Test Strip, 48 pcs in 6 plastic bottles, 8 pcs / bottle.
- Derivative reagent: 75mg
- Sample buffer: 15ml \*2
- Empty bottle: 1
- 1 manual
- NB Reader Fast 2 (for result reading, optional)

## 4. Reagents and consumables required but not provided

- 1M HCl
- 0.1M K<sub>2</sub>HPO<sub>4</sub>
- 1M NaOH
- Ethyl acetate
- Methanol
- n-Hexane
- Centrifuge tubes
- Glass beakers

## 5. Equipment required but not provided

- Stomacher/Homogenizer
- Vortex mixer
- Balance
- Centrifuge
- Rotary evaporators
- Pipette

## 6. Preparation of derivative reagent

Add 500ul methanol into the brown vial to dissolve the derivative reagent, then transfer the mixture into the bigger empty brown bottle, then add 9.5ml methanol, then the concentration of derivative reagent is 50mM.

## 7. Sample pretreatment

- Weigh 2.00±0.05 g of the homogenized sample into a 50 ml centrifuge tube, then add 4 ml deionized water, 0.5 ml 1M HCl and 0.2 ml derivative reagent successively, then vortex sample for 3 minutes.
- Incubate the sample at 60 °C for 15 minutes.
- Add 5 ml 0.1M K<sub>2</sub>HPO<sub>4</sub>, 0.4 ml 1M NaOH and 6 ml ethyl acetate successively, then vortex for 2 minutes.
- Centrifuge at 4000 rpm for 10 minutes.
- Take 3 ml of the upper yellow solution into a 15 ml centrifuge tube, dry the sample at 60 °C (the dried residue will be light red).
- Dissolve the dried residue in 0.5 ml n-Hexane, vortex for 1 minute.
- Then add 0.5 ml sample buffer, vortex for 1 minute and centrifuge at 4000 rpm for 1 minute.
- Take the lower layer solution (around 500 µl) as the sample solution.

## 8. Operations

- a) Please read the operating instructions carefully before the experiment. Bring the test kit and samples to room temperature.
- b) Remove the reagent bucket from the original packaging, then open it, remove the required number of microwell reagents and test strips, and mark them. Please use it as soon as possible within 60min. Immediately after removing the test reagent, cover the reagent lid.
- c) 200 µl of the sample solution was absorbed then tested into the microwells with a micropipette, slowly aspirate and mix well with the reagents in the microwells.
- d) After incubating for **5 min at room temperature (20-25 °C)**, insert the labeled test strip into the microwell, allow it to fully immerse into the solution.
- e) After incubating for **5 minutes at room temperature (20-25 °C)** again, the test strip was taken out and judged according to the schematic diagram, and the other conditions was judged to be invalid.

## 9. Result Determination

**Negative (-):** Both the C and T lines (T1, T2, T3, T4) are colored, and the T line is stronger than the C line, indicating that the concentration of nitrofurans metabolites in the sample is below the detection limit.

**Furaldone metabolites Positive (+):** T1 line color is the same as C line, T1 line color is weaker than C line or C line is colored and T1 line is not color.

**Nitrofurazone metabolites Positive (+):** T2 line color is the same as C line, T2 line color is weaker than C line or C line is colored and T2 line is not color.

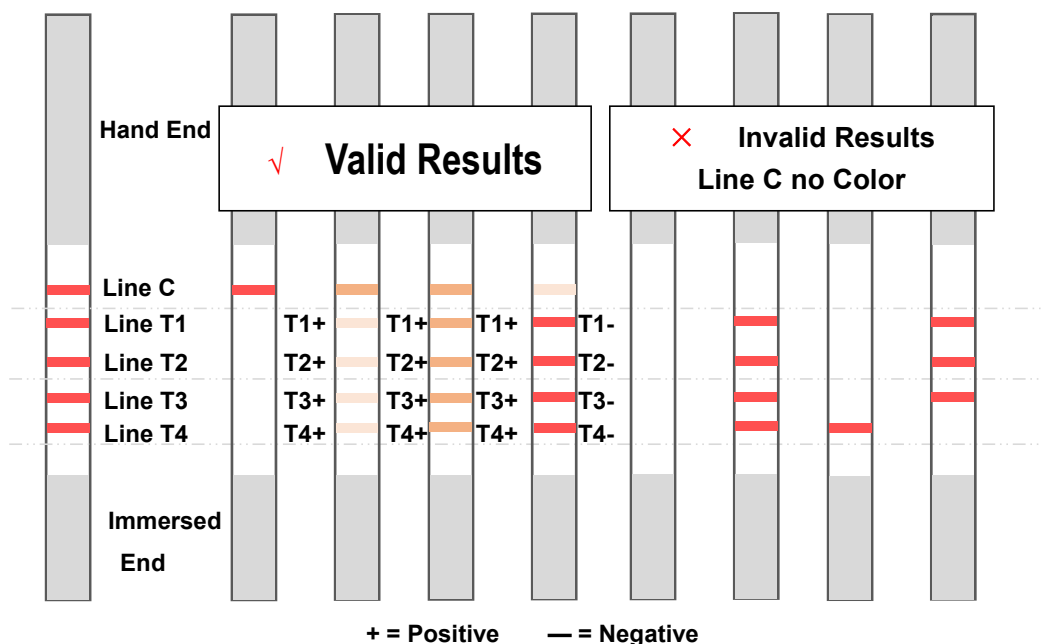
**Furazolidone metabolites Positive (+):** T3 line color is the same as C line, T3 line color is weaker than C line or C line is colored and T3 line is not color.

**Nitrofurantoin metabolites Positive (+):** T4 line color is the same as C line, T4 line color is weaker than C line or C line is colored and T4 line is not color.

**Invalid:** The C line does not appear, indicating that the incorrect operation process or the test strip has deteriorated. In this case, read the instruction carefully and retest with a new test strip.

If the test strip needs to be recorded, please cut off the lower sponge pad immediately after the interpretation and dry it for archiving.

**Remarks:** In addition to the naked eye interpretation, you can use a special reader to make the result interpretation.



## 10. Specificity

The results are all negative when test sulfonamides, tetracyclines, aminoglycosides and florfenicol with the concentration of 500 µg/kg.

## 11. Storage

2-8°C in cool dark place, do not freeze. The kit is valid for 12 months. Lot No. and expired date are printed on the package.

## 12. Notice and Precautions for a successful experiment.

- Please test follow the operation steps. Do not touch the color zone of the strip.
- Immediately after the test reagent is removed, cover the reagent bucket lid. If you can't use 8 microwells at a time, immediately cover the remaining microwells with a microwell lid and put it back in the reagent bucket for sealed storage. When one bucket is used up, open another bucket to protect it from moisture.
- Do not mix test strips and microwell reagents with different batch numbers.
- This test strip is a one-off product and should not be reused.
- The test results of this product are for reference only. If you need to confirm, please refer to the relevant national standard methods using LC/MS/MS.

**Ring Biotechnology Co., Ltd**

Add: Building 3, Zhongtongtai TechnoPark, No. 11, Kechuang 14th St, Beijing 100176, CHINA

E-mail: [info@ringbio.com](mailto:info@ringbio.com) Web: [www.ringbio.com](http://www.ringbio.com) Tel: +86-10-56242030