

Nitrofurazone is an antimicrobial organic compound belonging to the nitrofurans class. Nitrofurazone has demonstrated clear evidence to be mutagenic and carcinogenic during animal studies, the use of nitrofurazone, or related compounds, in animals raised for human consumption has been strictly banned.

1. Application

This kit can be used to qualitatively detect nitrofurazone metabolite in livestock (pork, beef, mutton), poultry (chicken), shrimp and fish.

2. Detection Limit (LOD) in shrimp sample

0.4-0.5 ppb

3. Kit components

- Test Strip, 16 pcs in 2 plastic bottles, 8 pcs / bottle.
- Derivative reagent: 75mg (Add 500ul of methanol into the brown vial to dissolve, then transfer the mixture into the bigger empty brown bottle, then add 9.5ml methanol, the concentration of derivative reagent is 50mM.)
- Reconstitution fluid: 10ml
- Empty bottle
- 1 manual

4. Reagents required but not provided

- 1M HCl
- 1M K₂HPO₄
- 1M NaOH
- Ethyl acetate
- n-Hexane

5. Sample pretreatment

- Remove the skin, take the meat, then treat it by a homogenizer.
- Weight **1.00±0.05g** homogenized sample into a **15ml** centrifuge tube, then add **2ml** deionized water, **0.25ml** HCl and **0.1ml** derivative reagent successively, then vortex sample for **3min**.
- Incubate the sample at **60°C** for **15min**.
- **Livestock&poultry:** Add **2.5ml** K₂HPO₄, **0.2ml** NaOH and **5ml** ethyl acetate successively, vortex for **2min**.
Shrimp: Add **2.5ml** K₂HPO₄, **0.2ml** NaOH and **3ml** ethyl acetate successively, then vortex for **2min**.
Fish: Add **2.5ml** K₂HPO₄, **0.2ml** NaOH and **4ml** ethyl acetate successively, then vortex for **2min**.
- Centrifuge at **4000rpm** for **10min**.
- **Livestock&poultry:** Take **2.5ml** upper yellow solution into a **4ml** centrifuge tube, dry the sample at **60°C** (the dried residue will be yellow green).
Shrimp: Take **1.5ml** upper yellow solution into a **4ml** centrifuge tube, dry the sample at **60°C** (the dried residue will be light red or light yellow).
Fish: Take **1.5ml** upper yellow solution into a **4ml** centrifuge tube, dry the sample at **60°C** (the dried residue will be light yellow green).
- Dissolve the dried residue in **0.25ml** n-hexane, vortex for **1min**.
- Then add **1ml** reconstitution fluid, vortex for **1min** and centrifuge at **4000rpm** for **1min**.
- Take the lower layer solution (around **750µl**) as the sample solution.

6. Operations

- a) Please read the instructions carefully before 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) Absorb 200µl sample solution into the microwells with a micropipette, slowly aspirate and mix well with the reagents in the microwells.
- d) Incubate for **5min at room temperature (20-25 °C)**, insert test strip into the microwell, allow it to fully immerse into the solution.
- e) Incubate for **5min 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.

7. Result Determination

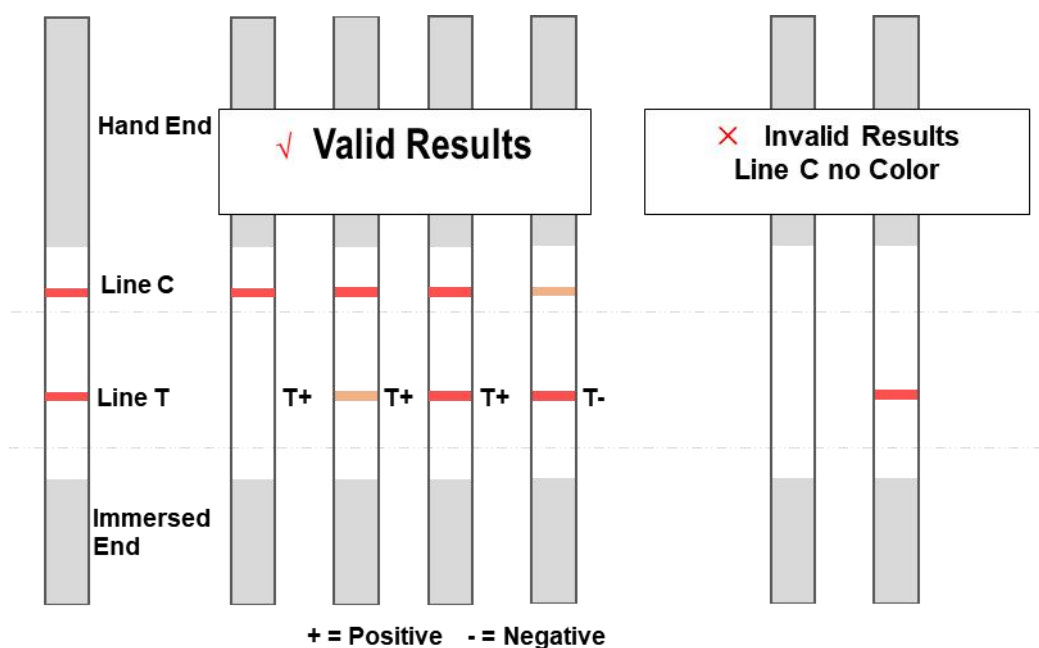
Negative (-): Both the C and T lines are colored, and the T line is stronger than the C line, indicating that the concentration of nitrofurazone metabolite in the sample is below the detection limit.

Positive (+): T line color is the same as C line, T line color is weaker than C line or C line is colored and T line is not color, which means that the concentration of nitrofurazone metabolite in the sample is equal to or higher than the detection limit.

Invalid: The C line does not appear, indicating that the incorrect operation process or the test strip has deteriorated. In this case, read the instructions 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 reader to make the result interpretation.



8. Specificity

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

9. Storage

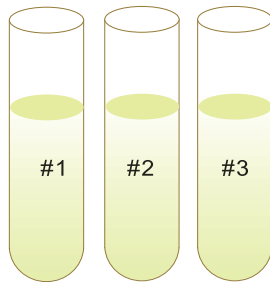
2-8°C in cool dark place, do not freeze. The kit is valid for 12 months. Lot No. and expired date are printed on the package.

10. Notice and Precautions for a successful experiment.

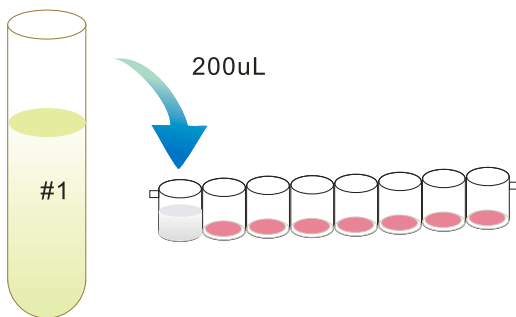
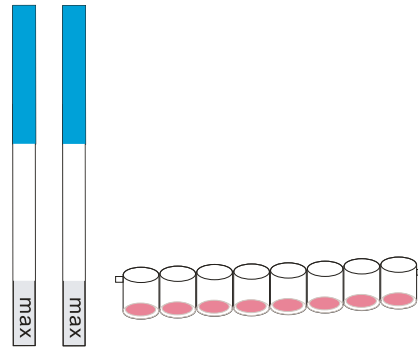
- Please test follow the operation steps. Do not touch the color zone of the strip.
- Immediately after the test reagent is removed, cover the reagent bucket lid. If you can't use 8 microwells at a time, immediately cover the remaining microwells with a microwell lid and put it back in the reagent bucket 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.

Schematic Assay Steps

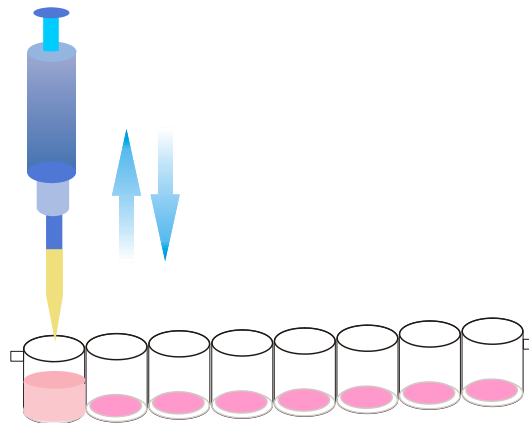
1. Bring all test samples to room temperature; number them to keep record.



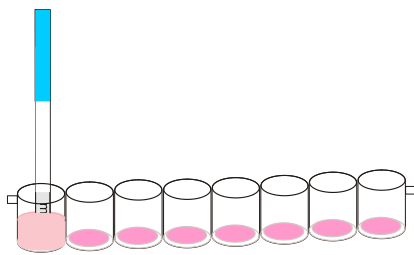
2. Take test kit according to your sample number and also number the kit wells to keep record and consistency.



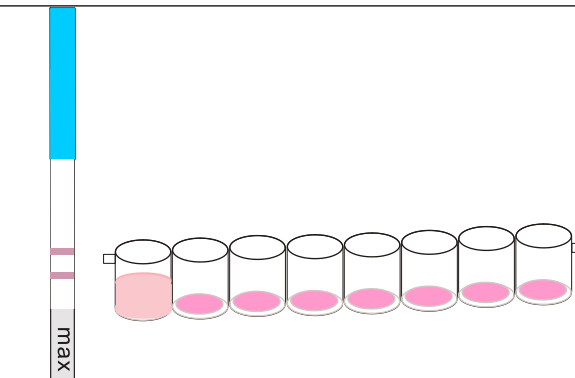
3. Take 200ul sample into the wells using pipet. You can also then put the well into the well holder to avoid sample spill.



4. Absorb up and down for 5 times to mix sample with reagent completely. Start the timer when the mixture is pink. **Incubate for 5 min at room temperature.**



5. Insert the "Immersed" end of the strip into the mixture; **Incubate for 5min at room temperature again.**



6. Take out the strip; judge the result according to **kit instruction.**

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