

Furazolidone is a nitrofurantoin antibacterial agent and monoamine oxidase inhibitor. As a veterinary medicine, furazolidone has been used in aquaculture. Since furazolidone is a nitrofurantoin antibiotic, its use in food animals is currently prohibited by the FDA under the Animal Medicinal Drug Use Clarification Act, 1994.

This product utilizes the high affinity of monoclonal antibody against furazolidone metabolite, which can easily identify its contamination in milk without any instrument.

1. Detection Limit (LOD)

0.2-0.5ppb

2. Kit components

- Test Strip, 48 pcs in 6 plastic bottles, 8 pcs / bottle.
- Derivative reagent, 75mg
- Kit insert
- Reconstitution fluid, 15ml*2

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

3. Reagents required but not provided

- 1M HCl
- 1M NaOH
- n-Hexane
- 0.1M K₂HPO₄
- Ethyl acetate

4. Sample pretreatment

- a) Remove the skin, take the meat, then treat it by a homogenizer.
- b) Weight 2.00±0.05g of the homogenized sample into a 50ml centrifuge tube, then add 4ml deionized water, 0.5ml 1M HCl and 0.2 ml derivative reagent successively, then vortex sample for 3 min.
- c) Incubate the sample at 60 °C for 15 min.
- d) Add 5ml K₂HPO₄, 0.4 ml NaOH and 6 ml ethyl acetate successively, then vortex for 2 min.
- e) Centrifuge at 6000rpm for 10min.
- f) Take 3ml of the upper yellow solution into a 15ml centrifuge tube, dry the sample at 60 °C (the dried residue will be light red).
- g) Dissolve the dried residue in 0.8 ml n-Hexane, vortex for 1 min.
- h) Then add 0.5ml reconstitution fluid, vortex for 1 min and centrifuge at 4000rpm for 1 min.
- i) Take the lower layer solution (around 500 µl) as the sample solution.

5. Operations

- a) Read the instructions before experiment. Bring the test kit and samples to room temperature.
- b) Take bottles needed from the kit package, take out required wells and strips, and make proper marks. Please use the test strips within 1h. Seal the cap of the bottles and store the unneeded kit.
- c) Take 200ul of the sample into the microwell, then repeatedly absorb up and down for 5 times to mix the sample with the reagent in the wells completely. The mixture should be pink, and then start the timer.
- d) Incubate for **5min at room temperature**, and then insert the test strip into the well with the "Immersed" end fully dipped in to the mixed reagent and sample.
- e) Incubate for **5min at room temperature** again. Take out the strip; determine the result according to **Part 6**.

6. Result Determination

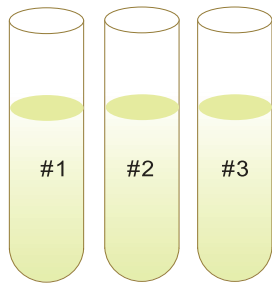
Positive (+): Line C is red, line T has no color or the color is lighter than or the same as Line C. If no line T, the result is also positive.

Negative (-): Line T and Line C are both red, and color of Line T is deeper than Line C.

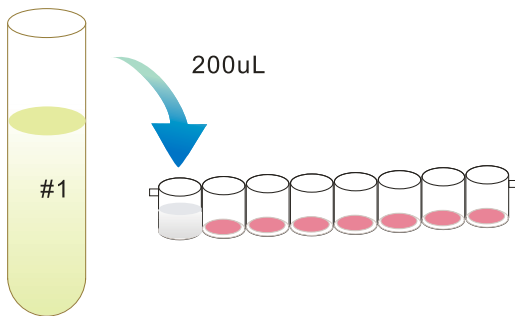
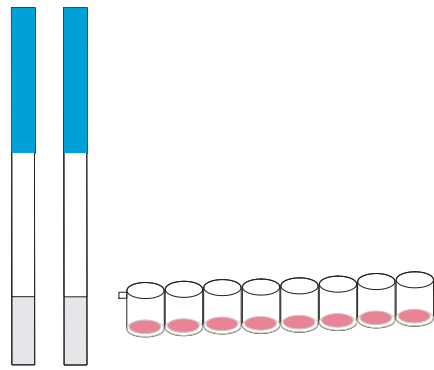
Invalid (x): Line C has no color. In this case, read the instructions carefully and retest with a new test strip.

Schematic Assay Steps

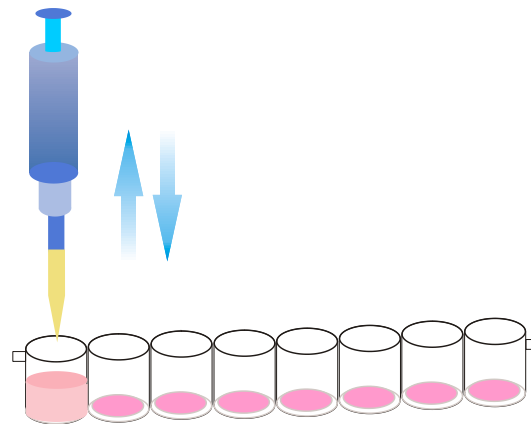
1. Bring all test samples to room temperature; number them to keep record.



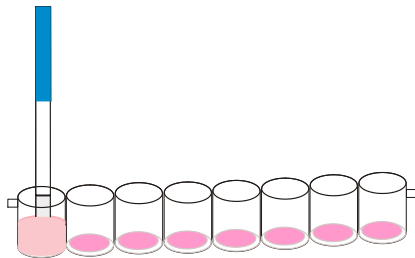
2. Take test kit according to your sample number and also number the kit wells to keep record and consistency.



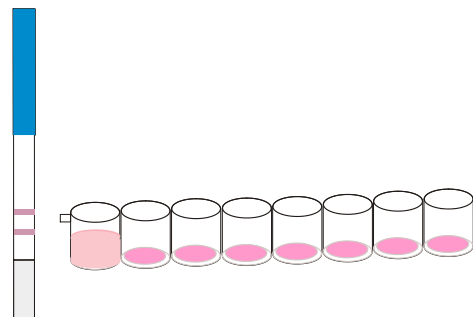
3. Take 200ul sample into the wells using pipet. You can also then put the well into the well holder to avoid sample spill.



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 5 min at room temperature again.**



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

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