Nitrofurazone Metabolite (SEM) Rapid Test Kit

Technical Manual

(GICA)

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1 Principle and Application

The test kit is used for detecting Nitrofurazone Metabolite (SEM) in various samples such as honey, tissue, and liver.

The kit is developed using the principle of competitive colloidal gold Immunochromatographic Assay (GICA). After the sample solution is added to sample hole, if SEM is present, it will bind with gold labeled antibodies, thereby preventing the labeled antibodies from binding to the SEM conjugates on the nitrocellulose membrane.

If the content of SEM in sample solution is less than detection limit, it will make the test ("T") line colored, and the result is negative. If the content is greater than detection limit, no color reaction will be produced, and the result is positive.

2 Technique Data

Kit Sensitivity: 1ppb (ppb=μg/kg)

The final detection limit for the sample must be calculated by multiplying the kit sensitivity by the dilution ratio used in sample processing.

Detection Limits:

Honey, tissue, casing, liver 0.5ppb

3 Kit Content

Package specification	20T/Kit
Test device (with disposable dropper)	20
Derivatization Reagent	10mL×2
Sample reconstitution buffer	30mL×1
Instruction	1

4 Materials Required but Not Supplied

Equipment: grinder (for crushing solid samples), vortex mixer (for shake and mix), graduated transfer pipette, and balance with a division value of 0.01 g, nitrogen evaporator, water bath.

Micropipettes: single-channel (20-200μL and 100-1000μL)

Reagents: Ethyl acetate, n-hexane, NaOH, concentrated HCl, $K_2HPO_4 \cdot 3H_2O$.

5 Sample Pre-treatment

5.1 Instructions

Labware must be clean. Use disposable pipette tips to avoid contamination of interference results.

5.2 Solution preparation before sample pre-treatment

Solution 1: 0.5M K_2HPO_4 Solution

Weigh 11.4g of $K_2HPO_4 \cdot 3H_2O$, dissolve in deionized water, and make up to 100mL.

Solution 2: 1M HCl Solution

Add 8.6mL of concentrated HCl to deionized water and make up to 100mL.

Solution 3: 1M NaOH Solution

Weigh 4g of NaOH, dissolve in deionized water, and make up to 100mL.

5.3 Sample pretreatment step:

5.3.1 Animal Tissue, Honey, Intestinal Casing, Liver, etc.:

For Fish, Shrimp, Crab, and Other Tissue Samples:

Remove the skin and select parts with minimal fat content. Use a grinder to homogenize the sample.

For Pork Tissue Samples:

Includes lean meat, intestinal casing, liver, lungs, and kidneys.

Samples must be tested immediately; if not possible, store at 2-8°C for up to 24 hours.

Prefer using non-frozen tissue samples for pre-treatment experiments.

For Honey Samples:

For non-crystallized samples, stir thoroughly to mix.

For crystallized samples, seal and warm in a water bath at 60°C-80°C until fully melted, then stir to mix.

(1)Weigh 2±0.05g of the homogenized sample into a centrifuge tube. Add 4mL of deionized water, 0.5mL of 1M HCl solution, and 600μL of derivatization reagent. Vortex for 5 minutes.

(2)Incubate the mixture in a 65°C water bath for 30 minutes.

(3)Add 1mL of 0.5M K_2HPO_4 Solution, 0.4mL of 1M NaOH solution, and 5mL of ethyl acetate.

(4)Centrifuge the mixture at room temperature at 4000 rpm for 5 minutes.

(5)Transfer 2.5mL of the upper organic layer to another centrifuge tube. Evaporate the solvent to dryness under nitrogen or air at 50-60°C.

(6)Dissolve the residue in 1mL of n-hexane. Add 0.5mL of the sample reconstitution buffer and vortex thoroughly for 30 seconds.

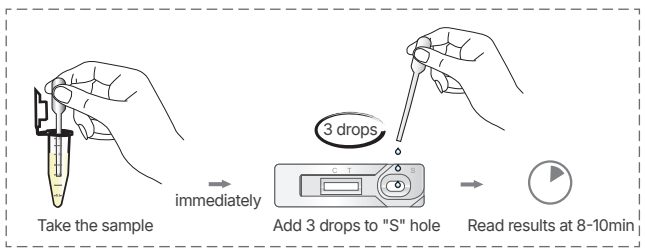
(7)Centrifuge at room temperature at 4000 rpm for 5 minutes. The upper layer is n-hexane; take the lower sample layer as the **sample solution.**

6 Test Steps

(1)Tear the foil pouch, take out of the test card, and put it on a flat, clean work surface.

(2)Pipette the prepared sample solution with the provided dropper, then add 3 drops (approximately 60μL) vertically and slowly (Avoid the generation of bubbles) into the sample hole(“S”).

(3)Read the result at room temperature in 8 to 10 minutes. Results over 10 minutes can only be used as reference.

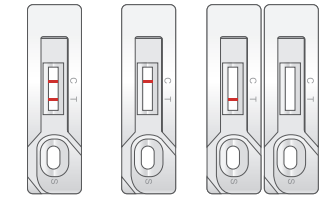


7 Results Judgement

Negative: Test(“T”) line and control(“C”) line both appear in the result window. It indicates that the concentration of ENR in the sample is below the detection limit, or absent.

Positive: Only control(“C”) line appears in the result window. It indicates that the concentration of ENR in the sample is above the detection limit.

Invalid: If the control(“C”) line does not appear, the result might be considered invalid.



8 Notice

8.1 Don’t use the expired or damaged products.

8.2 When the test card is taken out of the refrigerator, it should be restored to the room temperature and then opened. The opened test card should be used as soon as possible to avoid failure after being affected by moisture.

8.3 Avoid touching the white nitrocellulose membrane in the middle of the detection card.

8.4 In order to avoid cross-contamination, the droppers cannot pipet another Solution after pipetting one.

8.5 The sample solution to be examined needs to be clear and free of turbid particles. Otherwise, it is prone to lead to blockage, non-obvious color development and other abnormalities, affecting the determination of the experimental results.

9 Storage Conditions

The kit shall be stored at 2°C to 30°C (35.6°F to 86°F) in dry environment.

Shelf life: 12 months. The date of manufacture is presented in the label of the box.