Tetracyclines (TCs) Rapid Test Kit

Technical Manual

(GICA)

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1 Principle and Application

The test kit is used for the detection of tetracycline-class drugs (Tetracyclines, TCs) such as raw milk, tissue, egg and honey.

The tetracycline-class drugs are broad-spectrum antibiotics, primarily including tetracycline, doxycycline, chlortetracycline, and oxytetracycline, among others.

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

If the content of TCs in the sample solution is less than detection limit, it will make the test ("T") line colored (The color is consistent with the control line or deeper) and the result is negative. If the content is greater than detection limit, no color reaction will be produced (or color is lighter than the control line) and the result is positive.

2 Technique Data

Kit Sensitivity: 10ppb (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.

Limit of detection (LOD):

Fresh milk 10ppb

Poultry egg 60ppb

Honey 50ppb

Tissue:

LOD / Sample	Tetracycline (ppb)	Oxytetracycline (ppb)	Chlortetracycline (ppb)	Doxycycline (ppb)
Pork, fish, shrimp	10	10	1000	1000
Beef	20	20	2000	2000

3 Kit Content

Package specification	20T/Kit
Test device (with gold-labeled well and dropper)	20
Extraction Solution.	30mL×2
TCs Reconstitution Buffer	10mL×1
Instruction	1

4 Materials Required but Not Supplied

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

Micropipettes: single-channel 20 to 200μL and 100 to1000μL.

5 Sample Pre-treatment

Please note that the labware must be clean. Use disposable droppers to avoid contamination of interference results.

5.1 For Milk sample:

Fresh milk can be tested directly as the Test Fluid.

The frozen milk is obviously granules, which is easy to cause the liquid to fail to reach the control("C") line. The sample should be heated to 40-50°C using a constant temperature device until fully dissolved. What we get is the Test Fluid.

Reminder:

1)The fresh milk sample can be stored at 2-8°C for 24 hours to prevent invalidation or contamination if not assayed immediately. Return to room temperature before testing.

2)The testing is intended solely for raw milk; testing processed milk is not recommended.

3)Repeated freezing and thawing of raw milk can lead to deterioration, thereby affecting the experimental results.

5.2 For Tissue sample:

For shrimp meat samples, conduct a 5-minute water-bath at 80 °C first before the next operation. Other tissue samples can directly proceed to the next step without water-bath.

1)Take a certain amount of defatted and peeled samples and homogenize them with a grinder.

2)Weigh 4g ± 0.1g of the homogenized sample and put it into a 15mL centrifuge tube.

3)Add 2mL of Extraction Solution.

4)Vortex vigorously for 1 minute; Centrifuge at over 4000 rpm for 5 minutes, and the supernatant will be the

sample solution. Before testing, dilute the sample solution with TCs Reconstitution Buffer in different ratios using a 1.5 mL centrifuge tube. The resulting mixture is the **Test Fluid** (sample solution + TCs Reconstitution Buffer).

Dilution Method \ Sample	beef, fish, and shrimp	pork
Test Fluid	200μL of sample solution+200μL of TCs Reconstitution Buffer	No dilution needed. Use sample solution directly as Test Fluid.

5.3 For honey sample:

For non-crystallized honey samples, stir directly until evenly mixed. For crystallized samples, place in a sealed container and immerse in a water bath at 60°C. Once fully melted, stir until homogeneous.

1)Weigh 0.2g of honey sample and put it into a 1.5mL centrifuge tube.

2)Add 0.8 mL of TCs reconstitution buffer. Then use the provided dropper to wash down the inner wall of the tube with the added solution to ensure thorough mixing. The resulting mixture is the **Test Fluid.**

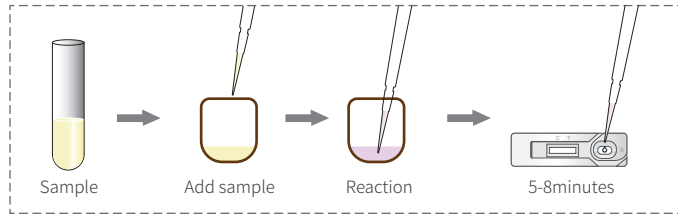
6 Test Steps

Restore the test kit and samples to room temperature before testing.

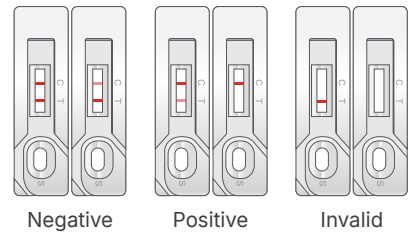
1)Tear the foil pouch, take out of the test card, gold-labeled well and dropper. Then put them on a flat, clean work surface.

2)Using the provided dropper, pipette 6 drops of Test Fluid (or transfer 150μL using a micropipette) vertically and slowly into the gold-labeled well. Prevent air bubbles during pipetting. Gently pipette up and down with the provided dropper (or a micropipette) for 30 seconds to completely dissolve the red substance at the bottom of the gold-labeled well. After allow it to stand for 2 minutes, draw the entire solution from the gold-labeled well and add them to the sample hole (“S”) on the test card and start the timer.

3)Determine the results in 5 to 8 minutes at room temperature.



7 Results Judgement



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

Positive: In the result window, the control(“C”) line appears, while the Test(“T”) line does not appear or appears lighter in color than the control(“C”) line. It indicates that the concentration of OTA 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.