

## Instructions for Use

Version: 1.0.1  
Revision date: 23-Mar-23

### Sulfonamides Rapid Test Kit

**Catalog No.:** abx092047

**Size:** 50 tests

**Sensitivity:** 20 ppb (ng/ml)

**Detection Limit:** Milk – 40 ppb; Honey – 20 ppb; Muscle – 5 ppb.

**Storage:** Store all reagents at 2 – 30 °C. Keep dry.

**Application:** For the qualitative detection of Sulfonamides in milk, honey, and muscle tissue samples.

#### Introduction and assay principle

Abbexa's Sulfonamides Rapid Test Kit is based on the gold immuno-chromatography assay (GICA) principle, using a competitive inhibition protocol. Any Sulfonamides present in the samples combine with the colloidal gold particle-labelled Sulfonamides antibody in the cassette well. When the concentration of Sulfonamides in the sample is more than the detection limit, the antibodies' binding sites are saturated, and so they cannot bind the Sulfonamides conjugate present on the detection membrane. Little to no color change is observed, and the result is positive. When the concentration of Sulfonamides in the sample solution is less than the detection limit, there is a strong color change in the detection line and the result is negative.

#### Kit Components

- Test cassettes with pipettes: 50
- Sample Buffer: 1 vial

#### Material Required But Not Provided

- Timer
- Pipette and pipette tips
- Concentrated (~12 M) HCl, or 0.5 M HCl Solution
- Ethyl acetate
- N-hexane
- Solid sodium hydroxide, or 0.2 M NaOH Solution
- Water bath or nitrogen evaporator
- Deionized water
- Centrifuge and microcentrifuge tubes
- Oscillator

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### Solution preparation

- **0.5 M HCl Working Solution:** Dilute 4.3 ml of concentrated HCl with 100 ml deionized water. Mix thoroughly.
- **0.2 M NaOH Working Solution:** Dilute 0.8 g powdered NaOH with 100 ml deionized water. Mix thoroughly to ensure the NaOH solid has fully dissolved.

### Sample preparation

- **Milk:** Dilute the milk with deionized water in a 1:1 ratio. Mix fully.
- **Honey:** Ensure the sample is homogenous; either homogenize the sample by hand, or by ultrasonication. Carefully weigh 1 g of homogenate into a microcentrifuge tube. Add 1 ml of the 0.5 M HCl Working Solution, and stand for 30 minutes at 37°C. Add 2.5 ml of the 0.2 M NaOH Working Solution, then 4 ml of Ethyl acetate, and oscillate for 5 minutes. Centrifuge at 4000 rpm for 10 minutes. Take 2 ml of the upper liquid layer and dry at 60°C with a water bath or nitrogen evaporator. Add 0.5 ml of Sample Buffer to dissolve the residue, then analyze the resulting solution.
- **Muscle:** Remove any skin, fat, or bone from the sample. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Carefully weigh 4 g of homogenate into a large centrifuge tube. Add 2 ml of Deionized water, and oscillate strongly until the sample has formed a homogenous paste. Add 4 ml of Ethyl acetate, and oscillate for 5 minutes. Centrifuge at 4000 rpm for 5 minutes. Take 2 ml of the upper liquid layer and dry at 60°C with a water bath or nitrogen evaporator. Add 0.5 ml of Sample Buffer to dissolve the residue, then analyze the resulting solution.

### Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- **For muscle tissues with high fat content,** add 1 – 2 ml of N-hexane after drying, then oscillate and mix fully to emulsify any large fat residues. Then, add 0.5 ml of Sample Buffer to dissolve the remaining homogenate, mix fully, and stand for 5 minutes. The solution will separate into two distinct phases; carefully take the lower layer for analysis.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

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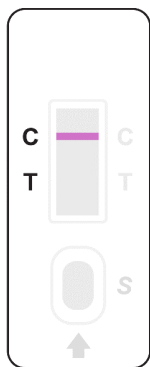
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### Assay procedure

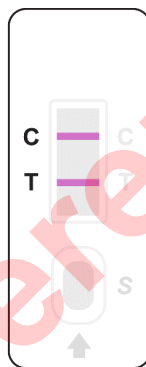
1. Take a test cassette and lay it flat on a clean table. Using the provided pipette, slowly and vertically add 2-3 drops (approximately 60 µl) of prepared sample to the sample well on the test cassette. Avoid foaming.
2. Leave at room temperature for 8 – 10 minutes, then analyze the result.

### Results analysis

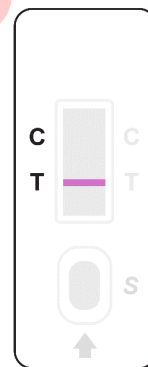
- **Positive result:** A colored line is observed in the control (C) section.
- **Negative result:** A colored line is observed in both the test (T) and control (C) sections.
- **Invalid result:** A colored line is observed in test (T) section but not the control (C) section, or no lines are observed.



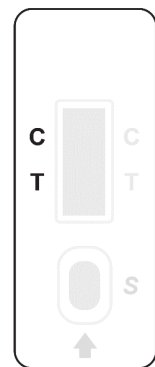
**Positive**



**Negative**



**Invalid**



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### Notes

1. The test cassettes should be brought to room temperature before use.
2. After opening the aluminum foil, use the test cassette as soon as possible.
3. Samples should be clear with no visible particles, turbidity or bacterial pollution.
4. Do not mix or re-use the disposable pipettes to avoid cross-contamination.
5. Avoid touching the cassette membrane through the sample well or test result window.
6. This kit is for qualitative detection of Sulfonamides in milk, honey, and muscle tissue samples. For other sample types, a preliminary experiment is recommended to determine compatibility with this kit. Positive samples can be tested with another method (e.g. HPLC, LC/MS) for quantitative results.
7. This kit is for research use only and the results are for reference only.
8. It is recommended to use this kit in conjunction with another detection method.
9. All waste should be disposed appropriately. Please note that you may need to follow special waste disposal procedures for infectious samples. Please check local disposal regulations.

The detection limits for various sulfonamides are provided below:

Sulfonamide	Sensitivity (ppb)	Sulfonamide	Sensitivity (ppb)
Sulfamethazine (SM2)	5	Sulfamethythiadiazole (SMT)	3
Sulfamonomethoxine (SMM)	0.8	Sulfaclozine (Esb3)	7.5
Aristebon (SMD)	1	Sulfathiazole (ST)	9
Sulfadimoxine (SDM')	1.5	Sulfachlorpyridazine (SCPA)	9
Sulfamerazine (SM1)	2	Sulfamethoxyypyridazine (SMP)	9
Sulfadiazine (SD/SDZ)	4	Sulfadimethoxine (SDT)	35
Sulfadimetine (SM2')	2.5	Sulfaquinoxaline (SQX)	35
Sulfadimethoxine (SDM)	3	Sulfasoxazole (SIZ)	120
Sulfapirazinmetossina (SMZ)	120		