

**CAP (Chloramphenicol) Lateral Flow Assay Kit**

Catalog No: E-FS-C102

20T/40T/80T

<b>Version Number:</b>	V1.1
<b>Replace version:</b>	V1.0
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This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017

Email: [techsupport@elabscience.com](mailto:techsupport@elabscience.com)

Website: [www.elabscience.com](http://www.elabscience.com)

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

### Test principle

This kit uses the principle of Immunochromatography assay for the qualitative detection. It can detect Chloramphenicol (CAP) in samples, such as honey, muscle, etc. After adding the sample solution into the sample well of detection card, CAP in the sample solution combine with the gold-labelled antibody, so as to prevent the combining of gold-labelled antibody with CAP conjugate on the cellulose membrane. When the concentration of CAP in the sample solution is more than the detection limit, the detect line do not show color (or shows lighter color than control line) and the result is positive. When the concentration of CAP in the sample solution is less than the detection limit, the detect line show color (shows equal or darker color than control line) and the result is negative.

### Technical indicator

**Detection limit:** Water---0.3 ppb; Milk, Muscle, Honey, Egg---0.1 ppb

### Kits components

Item	Specifications
Detection Card (with disposable dropper)	40 T/kit
Reconstitution Buffer	2 vials
Reagent A	2 vials
Reagent B	2 vials
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

### Other materials required but not supplied

**Instruments:** Homogenizer, Nitrogen Evaporators, Water bath, Centrifuge, Graduated pipette, Balance (sensitivity 0.01g), Oscillators.

**High-precision transferpettor:** Single channel (20-200  $\mu$ L, 100-1000  $\mu$ L).

**Reagent:** Ethyl acetate, N-hexane, Acetonitrile,  $ZnSO_4 \cdot 7H_2O$ ,  $Na_2[Fe(CN)_5NO] \cdot 2H_2O$ .

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## Notes

1. FOR RESEARCH USE ONLY. Do not use product out of date or in a broken aluminum foil.
2. The detection card should be adjusted to room temperature after removed from the refrigerator before opening. The opening detection card should be used as soon as possible so as not to be invalid because of moisture.
3. Avoid of contacting the white membrane at the middle of the sample well.
4. The disposable dropper cannot be mixing to avoid the cross-contaminant.
5. The tested sample should be clear, no turbidity particle and no bacterial pollution, otherwise it is easy to result in abnormal phenomena such as obstruction, unobvious color, etc., which affect the judgment of the experiment result.
6. **Each reagent is optimized for use in the E-FS-C102. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-C102 with different lot number**
7. The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS, etc. can be used for quantitative confirmation.

## Storage and expiry date

**Storage:** Store at 2-30°C. With cool and dry environment.

**Expiry date:** expiration date is on the packing box.

## Sample pretreatment

Restore all reagents and samples to room temperature (25°C) before use.

### 1. Sample pretreatment Notice:

Experimental apparatus should be clean, and the disposable dropper should be disposable to avoid the experiment result be interfered by the contamination.

### 2. Solution preparation

*Please prepare solution according to the number of samples. Don't use up all components in the kit at once!*

#### Solution 1: Acetonitrile solution

Dilute **Acetonitrile** with deionized water. (Acetonitrile (V): Deionized water (V) = 84 : 16).

#### Solution 2: **0.36M Na<sub>2</sub>[Fe(CN)<sub>5</sub>NO]·2H<sub>2</sub>O**

Dissolve **Reagent B** with 5 mL of deionized water, vortex thoroughly to dissolve completely.

#### Solution 3: **1.04M ZnSO<sub>4</sub>·7H<sub>2</sub>O**

Dissolve **Reagent A** with 5 mL of deionized water, vortex thoroughly to dissolve completely.

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### 3. Sample pretreatment procedure:

#### 3.1 Pretreatment of water sample:

Detect directly without pretreatment (Slow drop, too fast speed may result in detection failure).

**Note: Detection limit: 0.3 ppb**

#### 3.2 Pretreatment of milk sample:

- (1) Take 5 mL of milk into the 15 mL centrifuge tube, centrifuge at 4000 g for 10 min at 15°C.
- (2) Discard the upper layer of fat and take 3 mL of the lower layer of skim milk into a new 15 mL centrifuge tube.
- (3) Add 150 µL of **0.36M Na<sub>2</sub>[Fe(CN)<sub>5</sub>NO]·2H<sub>2</sub>O** (Solution 2), Vortex for 30 s, then add 50 µL of **1.04M ZnSO<sub>4</sub>·7H<sub>2</sub>O** (Solution 3), Vortex for 30 s, centrifuge at 4000 g for 10 min at 15°C.
- (4) Take 2.2 mL of the supernatant to another 15 mL centrifuge tube, add 4 mL of **Ethyl acetate**, mix fully for 2 min, centrifuge at 4000 g for 10 min at room temperature (25±2°C).
- (5) Take 2 mL of upper liquid (organic phase) to another 15 mL centrifuge tube, and dry at 50-60°C with nitrogen evaporators or water bath. (Please do it in a ventilated environment.)
- (6) Dissolve the residual with 2 mL of **N-hexane**, then add 0.3 mL of **Reconstitution Buffer**, oscillate until dissolved fully. After stand for 5 min.
- (7) Take the lower layer liquid to analysis.

**Note: Detection limit: 0.1 ppb**

#### 3.3 Pretreatment of muscle (livestock, fish, shrimp), honey sample:

- (1) Remove the skin, bone and fat of fish, shrimp and livestock, homogenize with a homogenizer (exclude honey sample).
- (2) Weigh 2±0.05 g of homogenate muscle into 15 mL centrifuge tube.
- (3) Add 2 mL of deionized water and 2 mL of **Ethyl acetate**, oscillate and mix fully. Centrifuge at 4000 r/min at room temperature (20-25°C) for 5 min.
- (4) Take 1 mL of upper liquid (organic phase) to another 15 mL centrifuge tube, and dry at 50-60°C with nitrogen evaporators or water bath. (Please do it in a ventilated environment.)
- (5) Dissolve the residual with 2 mL of **N-hexane**, then add 0.3 mL of **Reconstitution Buffer**, oscillate until dissolved fully. After stand for 5 min.
- (6) Take the lower layer liquid to analysis.

**Note: Detection limit: 0.1 ppb**

### 3.4 Pretreatment of egg sample:

- (1) Weigh  $3 \pm 0.05$  g of homogenate sample into 50 mL centrifuge tube, add 9 mL of **Acetonitrile solution** (Solution 1), oscillate for 2 min and mix fully. Centrifuge at 4000 r/min for 10 min at 15°C.
- (2) Take 3 mL of upper liquid to another centrifuge tube, add 3 mL of deionized water and 4.5 mL of **Ethyl acetate**, oscillate for 1 min and mix fully. Centrifuge at 4000 r/min for 10 min at 15°C.
- (3) Take all upper liquid to another 15 mL centrifuge tube, and dry at 50-60°C with nitrogen evaporators or water bath.
- (4) Dissolve the residual with 2 mL of **N-hexane**, then add 0.3 mL of **Reconstitution Buffer**. Oscillate until dissolved fully. After stand for 5 min.
- (5) Take the lower layer liquid to analysis.

**Note: Detection limit: 0.1 ppb**

### Experiment procedure

1. Tear the aluminum foil bag of the detection card and take out the detection card, and put it on a smooth, clean table.
2. Take the prepared sample with the matching disposable dropper, add 3 drops (about 60  $\mu$ L) of sample to the sample well (S) vertically and slowly (Avoid foaming).
3. Incubate for 8 to 10 minutes and then judge the results immediately.

### Judgment of result

1. **Negative:** The control line region (C) show color, the test line region (T) shows equal or darker than line C. It indicates the content of CAP in the sample is lower than detection limit or the sample doesn't contain CAP.
2. **Positive:** The control line region (C) show color, the test line region (T) shows no color or lighter color than line C. It indicates the content of CAP in the sample is higher than detection limit.
3. **Invalid:** The control line region (C) shows no color. It indicates operation process is wrong or the test card is invalid.

