UC Biodevices

Immunoaffinity Column for Confirmatory Test of Chloramphenicol (IAC-CAP)

(Product Number: 5501C309)

（Chloramphenicol is toxic to human）

INSTRUCTION MANUAL

(v. 1.00)

1. Introduction

Chloramphenicol (CAP) is a broad-spectrum bacteriostatic antibiotic, obtained originally from the bacterium Streptomyces venezuelae. Due to potential side effects in humans, the drug is not recommended for the treatment of minor diseases, but is reserved for the treatment of serious infections. In veterinary medicine, CAP has been shown to be a highly effective, well-tolerated antibiotic; the potential side effects observed in humans have not been reported in animals. However, because of its toxicity in humans, the use of CAP in animal-derived foods, including honey and milk has been strictly regulated. The European Union (EU) has defined a maximum residue limit (MRL) for CAP in food of animal origin at a level of 0.3 μg/kg, while China has an MRL level of 0.5 μg/kg.

2. INTENDED USE

A simple and efficient extraction and purification procedure for chloramphenicol was developed by means of the immunoaffinity column (IAC-CAP) as a cleanup tool. Chloramphenicol content in Feeds, Honey, Milk, Urine, Aquatic Products and Animal Derived Food are cleaned up by IAC and determined by HPLC or LC/MS/MS. It is a fast, simple, safe and highly accurate method for quantitatively measuring CAP.

3. PRINCIPLE

Samples are prepared by mixing with an extraction solution, blending and filtering. The extract is then applied to the chloramphenicol immunoaffinity column bound with specific antibodies to chloramphenicol. At this stage, the chloramphenicol binds to the antibody on the column. The column is then washed with water to remove the impurities in immunoaffinity column. By passing methanol through the column, the chloramphenicol is removed from the antibody. This methanol solution can then be injected into HPLC or LC/MS/MS/MS system.

4. PREPARATION OF SOLUTIONS

4.1 acetonitrile /water(5:95,v/v)：Take 50mL acetonitrile dissolved in DI water 1000mL

4.2 pH7.4 PBS：

8.0 g NaCl

2.9 g Na_2HPO_4. $12H_2O$

0.24g KH_2PO_4

0.2 g KCl

dissolved in approximately 990 mL DI water and diluted to 1 liter with DI water

5. METHOD: IAC-CAP Test Procedure for Aquatic Products and Animal Derived Food （0-130ppb）

5. 1 Sample Extraction

5.1.1 Place 6.0 g of muscle tissue homogenate into 50mL polypropylene centrifuge tube.

5.1.2 Add 20 ml ethyl acetate into the tube.

5.1.3 Mix them at high speed for 1 minute with vortex mixer, then shake it for 30min with an orbital shaker.

5.1.4 Centrifuge it at 4000RPM for 5min.

5.1.5 Pipette 10 ml supernatant extract into a clean vessel. Dry down under a nitrogen stream at 50°C. Reconstitute with 20mL acetonitrile /water (5:95, v/v).

5.1.6 Filter reconstitute extract through glass microfiber filter and Collect filtrate in a clean container.

5.2 Affinity Chromatography:

5.2.1 Remove two end caps from IAC

5.2.2 Pass 10 ml of filtered extract (10 ml = 1.5g sample equivalent) sample through the column at a steady slow flow rate of about 1 drop per second.

5.2.3 After extract has completely passed through column, pass 10 ml of purified water through column at a flow rate of about 1-2 drops per second.

5.2.4 Elute IAC-CAP column at a flow rate of 1 drop per second with 1.0 ml HPLC grade methanol and collect it in a clean glass cuvette.

5.2.5 Inject into HPLC or LC/MS/MS

5.3 Limit of detection (HPLC):10ppb

6. METHOD: IAC-CAP Test Procedure for Milk and Powdered Milk（Milk:0-100ppb, Powdered Milk:0-1000ppb）

6.1 Powder Milk Sample Preparation :

6.1.1 Weigh 10g of powder milk and place into a 250 mL beaker.

6.1.2 Heat 100 mL purified water to 30-40℃.

6.1.3 Add 80 mL preheated water in small amounts into the milk powder.
6.1.4 Mix continually until a homogeneous mixture is obtained.
6.1.5 Transfer milk mixture into a 250 mL graduate cylinder, add 20mL of remaining preheated water, and cool it to room temperature. It is called as recovery milk.

6.2 Sample Extraction
6.2.1 Measure 8.0 mL of fluid milk or recovery milk and place into 50mL polypropylene centrifuge tube.
6.2.2 Add 20 ml ethyl acetate into tube.
6.2.3 Shake it for 30min with an orbital shaker.
6.2.4 Centrifuge it at 4000RPM for 5min.
6.2.5 Pipette 10 ml of supernatant extract into a clean vessel. Dry down under a nitrogen stream at 50°C. Reconstitute with 20mL acetonitrile /water (5:95, v/v).
6.2.6 Filter reconstitute extract through glass microfiber filter and Collect filtrate in a clean container.

6.3 Affinity Chromatography:
6.3.1 Remove two end caps from IAC
6.3.2 Pass 10 ml of filtered extract (10 ml = 2.0mL sample fluid milk or 0.2g powder milk equivalent) sample through the column at a steady slow flow rate of about 1 drop per second.
6.3.3 After extract has completely passed through column, pass 10 ml of purified water through column at a flow rate of about 1-2 drops per second.
6.3.4 Elute IAC-CAP column at a flow rate of 1 drop per second with 1.0 ml HPLC grade methanol and collect it in a clean glass cuvette.
6.3.5 Inject into HPLC or LC/MS/MS

7.0 METHOD: IAC-CAP Test procedure for Honey（0-80ppb）

7.1 Sample Extraction
7.1.1 Place 10.0 g of Honey into 50mL polypropylene centrifuge tube.
7.1.2 Add 20 ml ethyl acetate into the tube.
7.1.3 Mix them at high speed for 1 minute with vortex mixer, and then shake it for 30min with an orbital shaker.
7.1.4 Centrifuge it at 4000RPM for 5min
7.1.5 Pipette 10 ml supernatant extract into a clean vessel. Dry down under a nitrogen stream at 50°C. Reconstitute with 20mL acetonitrile /water (5:95, v/v).
7.1.6 Filter reconstitute extract through glass microfiber filter and Collect filtrate in a clean container.

7.2 Affinity Chromatography:

7.2.1 Remove two end caps from IAC

7.2.2 Pass 10 ml of filtered extract (10 ml = 2.5g sample equivalent) sample through the column at a steady slow flow rate of about 1 drop per second.

7.2.3 After extract has completely passed through column, pass 10 ml purified water through column at a flow rate of about 1-2 drops per second.

7.2.4 Elute IAC-CAP column at a flow rate of 1 drop per second with 1.0 ml HPLC grade methanol and collect it in a clean glass cuvette.

7.2.5 Inject into HPLC or LC/MS/MS

8.0 METHOD: IAC-CAP Test procedure for Urine （0-100ppb）

8. 1 Sample Extraction

8.1.1 Measure 20 mL of Urine into 50mL polypropylene centrifuge tube.

8.1.4 Centrifuge it at 4000RPM for 5min.

8.2.1 Pipette 5 ml of supernatant extract into a clean vessel.

8.2.2 Dilute extract with 20ml pH7.4 PBS. Mix it thoroughly.

8.2.3 Filter diluted extract through glass microfiber filter and Collect filtrate in a clean container.

8.2 Affinity Chromatography:

8.2.1 Remove two end caps from IAC

8.2.2 Pass 10 ml of filtered extract (10 ml = 2.0mL sample equivalent) sample through the column at a steady slow flow rate of about 1 drop per second.

8.2.3 After extract has completely passed through column, pass 10 ml purified water through column at a flow rate of about 1-2 drops per second.

8.2.4 Elute IAC-CAP column at a flow rate of 1 drop per second with 1.0 ml HPLC grade methanol and collect it in a clean glass cuvette.

8.2.5 Inject into HPLC or LC/MS/MS

9.0 METHOD: IAC-CAP Test procedure for Feeds（0-2000ppb）

9. 1 Sample Extraction

9.1.1 Place 10.0 g of Feeds into 100mL conical beaker

9.1.2 Add 50 ml acetonitrile /water (80:20,v/v) into the tube.

9.1.3 Shake for 30min with an orbital shaker.

9.1.4 Pour extract into fluted filter paper. Collect filtrate in a clean vessel

9.1.5 Pipette 2 ml supernatant extract into a clean vessel.

9.1.6 Dilute extract with 38ml pH7.4 PBS. Mix it well.

9.1.7 Filter diluted extract through glass microfiber filter and collect filtrate in a clean container.

9.2 Affinity Chromatography:

9.2.1 Remove two end caps from IAC

9.2.2 Pass 10 ml of filtered extract (10 ml = 0.1g sample equivalent) sample equivalent) through the column at a steady slow flow rate of about 1 drop per second.

9.2.3 After extract has completely passed through column, pass 10 ml purified water through column at a flow rate of about 1-2 drops per second.

9.2.4 Elute IAC-CAP column at a flow rate of 1 drop per second with 1.0 ml HPLC grade methanol and collect it in a clean glass cuvette.

9.2.5 Inject into HPLC or LC/MS/MS

10.0 HPLC Setup:

10.1 Column: Cloversil-C18,4.6×150mm（5um）

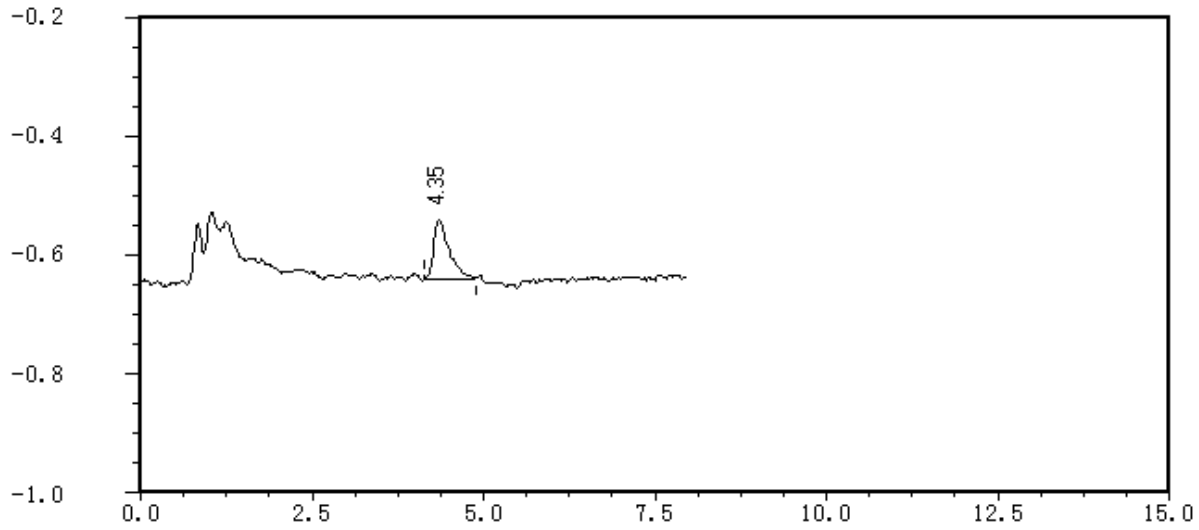
10.2 Flow rate: 0.8 mL/min.

10.3 Lamp: deuterium or mercury lamp

10.4 Detection: 278 nm

10.5 Sample loop: 50-200 µL

10.6 Mobile Phase: methanol:water (50:50) isocratic degassed



HPLC chromatogram of 20 ppb CAP contaminated Honey

11. NOTES

11.1 Storage: IAC-CAP and kits should be stored at 2-8℃. Do not freeze.

11.2 Shelf Life: IAC-CAP columns and kits are stable for 18 months from date of manufacture if stored at 2-8℃.

12. CONTACT INFORMATION

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