UC Biodevices

Immunoaffinity Column for Confirmatory Test of β-Agonists

(IAC-CLE/SAL)

(Product Number: 5501C202)

（ß-Agonists can cause life threat to human）

INSTRUCTION MANUAL

(v. 1.00)

1. Introduction

β-Agonists are synthetic derivatives of the naturally occurring catecholamines. It has been known that β-agonists are suitable for use as performance improvers within the field of livestock production; in particular, the meat/fat ratio in fattened animals can be improved or the growth may be accelerated. However, such compounds have not been approved in the EU for use as fattening adjuvants. In addition to lipolytic and anabolic effects, β-agonists have relaxing effects on non-striated musculature. Thus they can be used as antiasthmatic and tocolytic agents. It is possible that β-agonists residues, after use in illegal practice, may lead to a risk for consumers. Therefore there exists a prohibition of β -agonists use in food production.

2 INTENDED USE

A simple and efficient extraction and purification procedure for β-Agonists was developed by means of the immunoaffinity column (IAC-CLE/SAL/RAC) as a cleanup tool. β-Agonists content in Feeds, urine and Animal Derived Food are cleaned up by IAC and determined by HPLC or LC-MS. It is a fast, simple, safe and highly accurate method for quantitatively measuring β-Agonists (2 analogs)

2 kinds of β-Agonists

Clenbuterol（CLE）
Salbutamol（SAL）

3 PRINCIPLE

Samples are prepared by mixing with an extraction solution, blending and filtering. The extract is then applied to the β-Agonists immunoaffinity column bound with specific antibodies to β-Agonists. At this stage, the β-Agonists bind 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 β-Agonists are removed from the antibody. This methanol solution can then be injected into HPLC or LC-MS/MS system.

4 PREPARATION OF SOLUTIONS

4.1 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, diluted to 1 liter with DI water

4.2 0.05M Sodium acetate

6.80 Na_2Ac is dissolved in 900 mL purified water. Adjust to pH 5.2 with acetic acid and dilute to 1000 mL.

4.3 Glucuronidase/ Arylsulfatase： from Helix pomatia Type H-2, aqueous solution, ≥85,000units/mL (from sigma, Product Number: G0876)

5. METHOD: IAC-CLE/SAL Test Procedure for Aquatic Products and Animal Derived Food（0-200ppb）

5.1 Sample Extraction

5.1.1 Place 10.0g of muscle tissue homogenate into 50mL polypropylene centrifuge tube

5.1.2 Add to tube 10ml 0.05M Sodium acetate and 20µl Glucuronidase/ Arylsulfatase, incubate at 37℃ for 12h;

5.1.3 Add 15 ml acetonitrile. Shake for 90min with an orbital shaker

5.1.4 After centrifugation at 4000RPM for 10min, the supernatant was decanted into a clean tube

5.2 Extract Dilution and Filtration

5.2.1 Pipette 5 ml supernatant extract into a clean vessel.

5.2.2 Dilute extract with 45ml pH7.4 PBS. Mix well.

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

5.3 Affinity Chromatography:

5.3.1 Remove two end caps from IAC

5.3.2 Pass 10 ml of filtered extract (10 ml = 0.4g sample equivalent) through the column at a steady slow flow rate of about 1 drop per second.

5.3.3 After extract has completely passed through column, pass 10 ml distilled water through column at about 1-2 drops per second flow rate.

5.3.4 Elute column at flow rate of 1 drop per second with 2.0 ml HPLC grade methanol and collect in a clean glass cuvette.

5.3.5 Dry down eluate under an Nitrogen stream at 50℃. Reconstitute with 400µL mobile phase.

5.3.6 Inject into HPLC or LC-MS

6. NOTES

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

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

7. CONTACT INFORMATION

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