UC Biodevices

Immunoaffinity Column for Confirmatory Test of Zeranols

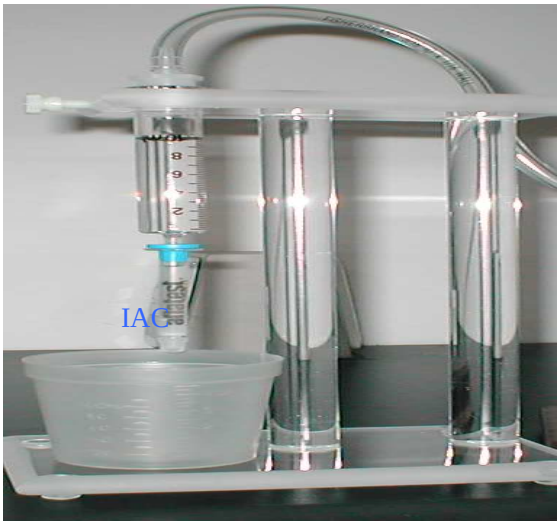
(IAC-ZER)

(Product Number: 5501C205)

（Zeranol causes human sexual function disorders and is potentially carcinogenic）

INSTRUCTION MANUAL

(v. 1.00)



1. Introduction

Zeranol (ZER) is a female hormone, which maintains secondary sexual characteristics and protein assimilation effect. It can be used for growth promotion of animals besides therapy, especially in cattle. It is now being inhibited for growth promotion in many countries for the obvious carcinogenicity.

2. INTENDED USE

A simple and efficient extraction and purification procedure for zeranols was developed by means of the immunoaffinity column (IAC-ZER) as a cleanup tool. Zeranols content in Feeds, 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 zeranols (6 analogs)

6 kinds of Zeranols

α-zearalanol/zeranol	β-zearalanol/β-zeranol
α-zearalenol	β-zearalenol
zearalanone	zearalenone

3. PRINCIPLE

Samples are prepared by mixing with an extraction solution, blending and filtering. The extract is then applied to the zeranols immunoaffinity column bound with specific antibodies to zeranols. At this stage, the zeranols binds to the antibody on the column. The column is then washed with water to rid the immunoaffinity column of impurities. By passing methanol through the column, the zeranols is 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 and diluted to 1 liter with DI water

4.2 1.0 M NaOH

40.0g NaOH is dissolved in 1 liter of DI water

4.3 0.05M Sodium acetate

6.80 Na_2Ac is dissolved in 900 mL DI water. Adjust to pH 4.8 with acetic acid and dilute to 1000 mL.

4.4 Glucuronidase/ Arylsulfatase： from Helix pomatia Type H-2, aqueous solution, ≥85,000units/mL

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

5. 1 Sample Extraction

5.1.1 Place 5.0 g 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 10 ml acetonitrile and 200ul 1.0 M NaOH into the tube.

5.1.4 Shake for 30min with an orbital shaker

5.1.5 After centrifugation at 4000RPM for 3min, the supernatant was decanted into a clean tube

5.2 Extract Dilution and Filtration

5.2.1 Pipette 10 ml supernatant extract into a clean vessel.

5.2.2 Dilute extract with 30ml 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 8 ml of filtered extract (8 ml = 0.5g 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 flow rate of about 1-2 drops per second.

5.3.4 Elute ZER 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 500µL mobile phase.

5.3.6 Inject into HPLC or LC/MS/MS

6. NOTES

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

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

7. CONTACT INFORMATION

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