Immunoaffinity Column of T-2 toxin (IAC-T-2)

Instruction Manual (C/N: IAC 108)

1. GENERAL

T-2 producing fungi commonly attack grains and can grow at temperatures from slightly above freezing to about 30℃ All domestic animals are susceptible to injury by dietary intake of T-2 in the range of a few ppm. In poultry, feed contaminated with 1.0 to 3.5 ppm of T-2 has produced lesions at the edges of the beaks, abnormal feathering in chicks, a drastic and sudden drop in egg production, eggs with thin shells, reduced weight gains, and mortality.

2. INTENDED USE

A simple and efficient extraction and purification procedure for T-2 toxin was developed by means of the immunoaffinity column (IAC-SEP® T-2) as a cleanup tool. T-2 toxin content in grain, food, feed, nuts, peanuts, soy sauce, vinegar, chili, pepper, medicinal herbs and wine samples 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 T-2 toxin.

3. PRINCIPLE

Samples are prepared by mixing with an extraction solution, blending and filtering.The extract is then applied to the T-2 toxin immunoaffinity column bound with specific antibodies to T-2 toxin. At this stage, the T-2 toxin bind to the antibody on the column.The column is then washed with water to remove the impurities.By passing methanol through the column, the T-2 toxin 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 **Extracting solution:**

Methanol-water（8+2,V/V）：80mL Methanol+20mLwater, mixing blending.

4.2 **pH7.0 PBS：**

8.0 g NaCl

1.44 g Na_2HPO_4. $12H_2O$

0.24g KH_2PO_4

0.2 g KCl

dissolve in approximately 990 mL purified water,adjust the pH to 7.0, bring to 1L with purified water.

4.3 **4-Dimethylaminopyridine Solution:** weigh 0.0325g of 4-dimethylaminopyridine in a 100mL volumetric flask ,bring to100mL with toluene. mixing blending.

4.4 **1-Nitronitrile solution:** weigh 0.030g 1-phthalonitrile in a 100mL volumetric flask，bring to 100mL with toluene. mixing blending.

The column capacity of IAC-T-2 (maximum adsorption amount of T-2 toxin) is 1500 ng, when T-2 toxin in sample more than the maximum adsorption amount, please reduced the volume into the detection range, then calculate the accurate content.

5. METHOD: IAC-T-2Test procedure for Rice, Corn, Wheat, Nuts, Spices, Peanuts Peanuts products, Vegetable oil, Feed.

5.1 Sample Extraction:

5.1.1 Weigh 25g sample with 5g NaCl and place in blender jar.

5.1.2 Add to jar 100mL methanol: water (80:20).

5.1.3 Cover blender jar and blend at high speed for 2 min.

5.1.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

5.2 Extract Dilution

5.2.1 Pipet or pour 10mL filtered extract into a clean vessel.

5.2.2 Dilute extract with 40mL of purified water. Mix well.

5.2.3 Filter dilute extract through glass microfibre filter into a clean vessel.

5.3 Column Chromatography

5.3.1 Pass 10mL filtered diluted extract (10mL = 0.5g sample equivalent) completely through IAC at a rate of about 1-2 drops/second until air comes through column.

5.3.2 Pass 10mL of purified water through the column at a rate of about 2 drops/second.

5.3.3 Repeat step 5.3.2 once more until air comes through the column.

5.3.4 Place glass cuvette under IAC and add 1.5mL HPLC grade methanol into glass syringe barrel.

5.3.5 Elute IAC at a rate of 1 drop/second by passing the methanol through the column and collecting all of the sample eluate (1.0mL) in a glass cuvette.

5.3.6 Dry down eluate under an Nitrogen stream at 50℃.

5.4 Derivatization Procedure

5.4.1 Add 50μL of 4-dimethylaminopyridine (DMAP) solution into the vial followed by the addition of 50 μL of 1-anthroyl cyanide (1-AN) reagent. Mix by vortex for 1 minute.

5.4.2 Leave to react for 15 minutes at 50℃. Cool mixture in ice for approximately 10 minutes.

5.4.3 Evaporate to dryness the entire volume of the mixture under nitrogen stream at approximately 50℃ and reconstitute with 1000 μL HPLC mobile phase. Inject 5-100μL into HPLC.

6. METHOD: IAC-T-2Test procedure for Soy sauce, Vinegar, Liquor.

6.1 Sample Extraction:

6.1.1 Weigh 25g sample into 100mL volumetric flask

6.1.2 Bring to 100mL with methanol and transfer it to a blender jar.

6.1.3 Cover blender jar and blend at high speed for 2 minute.

6.1.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

6.2 Extract Dilution

6.2.1 Pipet or pour 10 mL filtered extract into a clean vessel.

6.2.2 Dilute extract with 40 mL of pH7.0 PBS(4.2). Mix well.

6.2.3 Filter dilute extract through glass microfibre filter into a clean vessel.

6.3 Column Chromatography

6.3.1 Pass 10mL filtered diluted extract (10mL = 0.5g sample equivalent) completely through IAC

at a rate of about 1-2 drops/second until air comes through column.

6.3.2 Pass 10 mL of purified water through the column at a rate of about 2 drops/second.

6.3.3 Repeat step 6.3.2 once more until air comes through the column.

6.3.4 Place glass cuvette under IAC and add 1.5mL HPLC grade methanol into glass syringe barrel.

6.3.5 Elute IAC at a rate of 1 drop/second by passing the methanol through the column and collecting all of the sample eluate (1.0mL) in a glass cuvette.

6.3.6 Dry down eluate under an Nitrogen stream at 50℃.

6.4 Derivatization Procedure

6.4.1 Add 50μL of 4-dimethylaminopyridine (DMAP) solution into the vial followed by the addition of 50μL of 1-anthroyl cyanide (1-AN) reagent. Mix by vortex for 1 minute.

6.4.2 Leave to react for 15 min at 50℃. Cool mixture in ice for approximately 10 minutes.

6.4.3 Evaporate to dryness the entire volume of the mixture under nitrogen stream at approximately 50℃ and reconstitute with 1000 μL HPLC mobile phase. Inject 5-100μL into HPLC.

7. HPLC Set up 1(Derivatization):

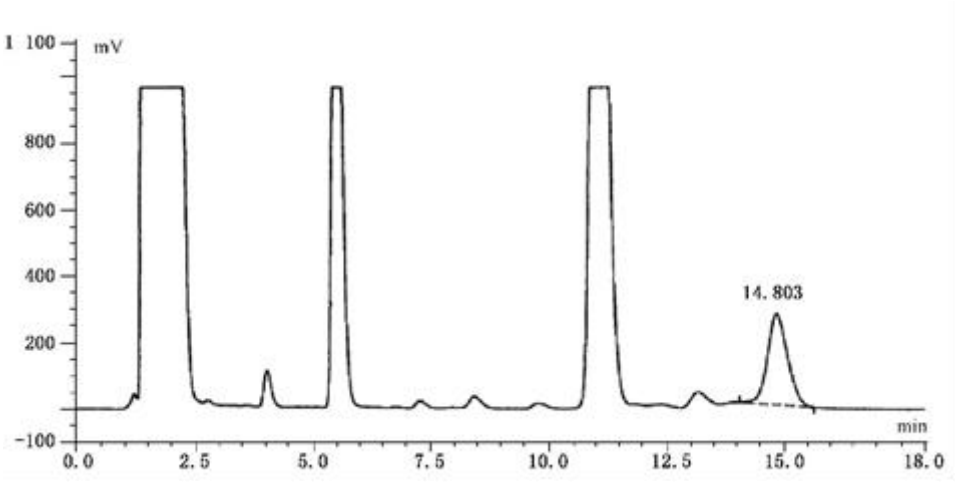
7.1 Column: Cloversil-C18,4.6×150mm（5um）or 4.6*250mm（5um）.

7.2 Flow rate: 0.8 mL/min.

7.3 Detector: Fluorescence detector Excitation wavelength: 381 nm, Emission wavelength:470 nm

7.4 Sample loop: 5-100 μL

7.5 Mobile Phase : acetonitrile -water（75+25,V/V）.



HPLC chromatogram of T-2 standard(Derivatization)

8. HPLC Set up2(No Derivatization):

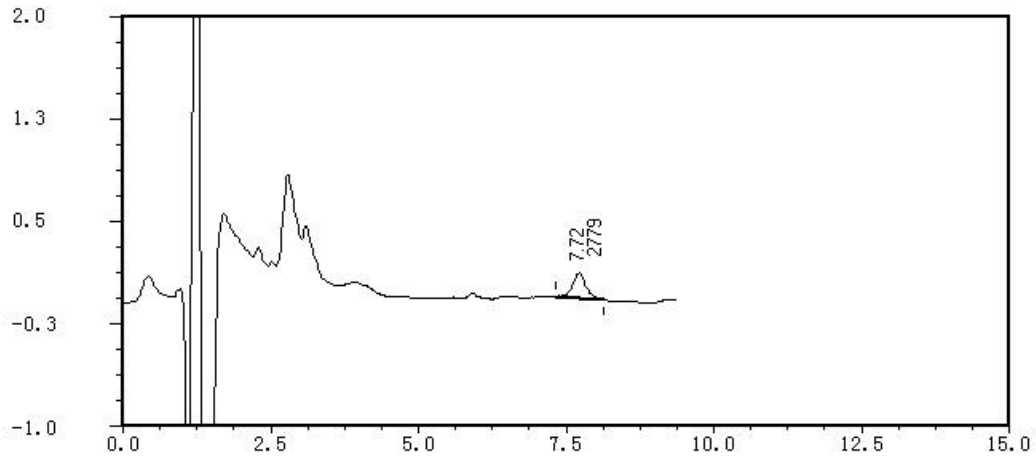
8.1 Column: Agilent-C18,4.6×250mm（5um）

8.2 Flow rate: 0.8 mL/min.

8.3 Detector: Ultraviolet detector:wavelength: 208 nm

8.4 Sample loop: 20-100μL

8.5 Mobile Phase : Methanol -water（55+45,V/V）.



HPLC chromatogram of T-2 standard (No Derivatization)

9. IMPORTANT NOTES

9.1 Storage: IAC-T-2 should be stored at 2-8 ºC. Do not freeze.

9.2 Shelf Life: IAC-T-2 columns and kits are stable for 18 months from date if stored at 2-8 ºC.

9.3 If Sample recycling test is needed, standard substance should be added to the sample before 2 hours or one night,otherwise, the recovery rate will be low.If standard substance recycling test is needed,make sure methanol concentration <25%,or the adsorption capacity of immunoaffinity column will be influenced.

9.4 If you want to modify the operating instructions of the operation steps, please contact with our technology department.