Immunoaffinity Column of AflatoxinB_1 (IAC-AFLA B1)

Instruction Manual (C/N: IAC 101)

1. GENERAL

Aflatoxin is a kind of fungus toxic metabolites. They mainly exist in the peanut, grains, nuts, seeds and some food, animal feed and other related products. Natural pollution of aflatoxin is given priority to aflatoxin B1,which is classified as first-degree carcinogen by WHO is the most toxic . It proved has great harm to human and animal liver, kidneys and other tissues and organs. Aflatoxin contamination can occur in plant growth, harvest and processing, storage, transportation in the process. The timely detection of pollution sources is the best way to prevent aflatoxin contamination.

2. INTENDED USE

A simple and efficient extraction and purification procedure for Aflatoxin was developed by means of the immunoaffinity column (IAC-SEP®AFLA-B_1) as a cleanup tool. AflatoxinB1 content in Grain, Food, Feeds, 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 aflatoxin-B_1.

3. PRINCIPLE

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

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

Methanol-water（7+3,V/V）：70mL Methanol+30mLwater，mixing blending.

Acetonitrile-water（9+1,V/V）：90mL Methanol+10mLwater，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 1 liter with purified water

4.3 0.1%Tween-20 pH7.0 PBS: Add 1.0mL Tween-20 into 1L pH 7.0 PBS, mixing blending.

4.4 0.5%Tween-20 pH7.0 PBS: Add 5.0mL Tween-20 into 1L pH 7.0 PBS, mixing blending.

4.5 Mobile Phase :Methanol-water（45+55,V/V）:450mL Methanol+550mLwater，mixing blending.

4.6 Standard solution: Dilute aflatoxin B1 stock solution with mobile phase.(2-8 ℃ storage, valid for 24 h)

The column capacity of IAC-SEP® AFLA-B1 (maximum adsorption amount of aflatoxinB1) is 400 ng, when aflatoxinB1 in sample more than the maximum adsorption amount, please reduced the volume into the detection range, then calculate the accurate content.

5. METHOD: IAC-AFLA B1 Test procedure for Rice, Corn, Wheat, Nuts, Spices, Peanuts Peanuts products, Vegetable oil， Chili, Pepper.

5.1 Sample Extraction:

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

5.1.2 Add to jar 125 mL methanol: water (70:30).

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

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 15 mL filtered extract into a clean vessel.

5.2.2 Dilute extract with 30 mL 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 15mL filtered diluted extract (15mL = 1g sample equivalent) completely through IAC at a rate of about 1-2 drops/second until air comes through column.

5.3.2 Pass 10 mL 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 1.0 mL 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 (1.0 mL) in a glass cuvette.

5.3.6 Add 1.0 mL of purified water to eluate. Mix well. Inject 5-100 µL into HPLC.

6. METHOD: IAC-AFLA B1 Test procedure for Soy sauce.

6.1 Sample Extraction:

6.1.1 Measure 20mL sample place in blender jar.

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

6.1.3 Cover blender jar and blend at high speed for 1 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 purified water. 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 = 0.4 ml 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 0.1%Tween-pH7.0 PBS through the column at a rate of about 2 drops/second.

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

6.3.4 Place glass cuvette under IAC and add 1.0 mL 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 (1.0 mL) in a glass cuvette.

6.3.6 Add 1.0 mL of purified water to eluate. Mix well. Inject 5-100 µL into HPLC.

7. METHOD: IAC-AFLA B1 Test procedure for Vinegar.

7.1 Sample Extraction:

7.1.1 Measure 5mL sample place in 50ml centrifuge tube.

7.1.2 Add to tube 20 mL pH7.0 PBS. Mix well.

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

7.2 Extract Dilution

7.2.1 Pipet or pour 20 mL filtered extract into a clean vessel.

7.2.2 Dilute extract with 20 mL of pH7.0 PBS. Mix well.

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

7.3 Column Chromatography

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

7.3.2 Pass 10 mL of 0.1%Tween-pH7.0 PBS through the column at a rate of about 2 drops/second.

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

7.3.4 Place glass cuvette under IAC and add 1.0 mL HPLC grade methanol into glass syringe barrel.

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

7.3.6 Add 1.0 mL of purified water to eluate. Mix well. Inject 5-100 µL into HPLC.

8. METHOD: IAC-AFLA B1 Test procedure for Feeds.

8.1 Sample Extraction:

8.1.1 Weigh 50g sample with 5g NaCl and place in blender jar.

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

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

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

8.2 Extract Dilution

8.2.1 Pipet or pour 5 mL filtered extract into a clean vessel.

8.2.2 Dilute extract with 45 mL of pH7.0 PBS. Mix well.

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

8.3 Column Chromatography

8.3.1 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.

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

8.3.3 Repeat step **8.3.2** once more until air comes through the column.

8.3.4 Place glass cuvette under IAC and add 1.0 mL HPLC grade methanol into glass syringe barrel.

8.3.5 Elute IAC at a rate of 1 drop/second by passing the methanol through the column and

collecting all of the sample eluate (1.0 mL) in a glass cuvette.

8.3.6 Add 1.0 mL of purified water to eluate. Mix well. Inject 5-100 µL into HPLC.

9. METHOD: IAC-AFLA B1 Test procedure for Medicinal herbs.

9.1 Sample Extraction:

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

9.1.2 Add to jar 125 mL Acetonitrile: water (90:10).

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

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

9.2 Extract Dilution

9.2.1 Pipet or pour 5 mL filtered extract into a clean vessel.

9.2.2 Dilute extract with 45 mL of 0.5%Tween-pH7.0 PBS. Mix well.

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

9.3 Column Chromatography

9.3.1 Pass 15mL filtered diluted extract (20mL = 0.4g sample equivalent) completely through IAC at a rate of about 1-2 drops/second until air comes through column.

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

9.3.3 Repeat step **9.3.2** once more until air comes through the column.

9.3.4 Place glass cuvette under IAC and add 1.0 mL HPLC grade methanol into glass syringe barrel.

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

9.3.6 Add 1.0 mL of purified water to eluate. Mix well. Inject 5-100 µL into HPLC.

10. HPLC Set up:

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

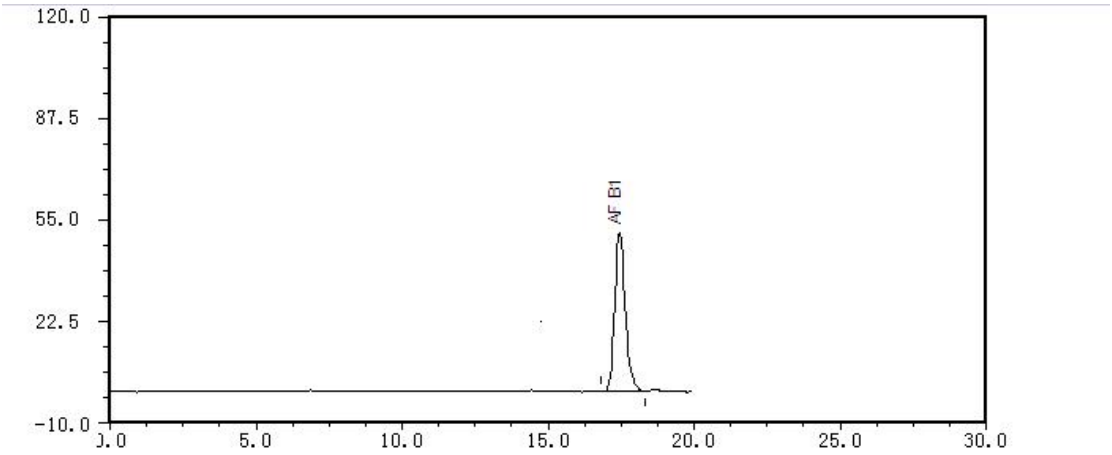
10.2 Flow rate: 0.8 mL/min.

10.3 Detector: Fluorescence detector Excitation wavelength: 360 nm, Emission wavelength:440 nm

10.4 Sample loop: 5-100 µL

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

10.6 Photochemical derivatization system



HPLC chromatogram of AFLA-B_1 standard

11. IMPORTANT NOTES

11.1 Storage: IAC-AFLA B_1 should be stored at 2-8 ºC. Do not freeze.

11.2 Shelf Life: IAC-AFLA B_1 columns and kits are stable for 18 months if stored at 2-8 ºC.

11.3 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.

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