

# Fumonisin Total Immunoaffinity Column

## 1. Product Code: CUS-C09C

## 2. Application

This product is used for Fumonisin Total purification in cereal and feed samples prior to HPLC, HPLC-MS/MS or Specialized Rapid Tester. It reduces matrix interference and improves analysis accuracy.

## 3. Measuring Principle

It is based on antigen-antibody binding reaction. Fumonisin Total monoclonal antibody is coupled to agarose gel material. After extracting, filtering and diluting Fumonisin Total in sample, sample extracting solution slowly passes through IAC. Fumonisin Total in sample extracting solution combines with specific monoclonal antibody, meanwhile, impurities are washed away. Finally, elute Fumonisin Total compound to get purified Fumonisin Total by using methanol.

## 4. Product Performance

Capacity:  $\geq 5000$  ng/vial

Rate: 85%~110%

Column Gel: **Agarose Gel**

Cross Reaction Rate:

FB1	100%
FB2	38%
FB3	79%

### Features:

- **Easy to elute**
- **High recovery rate**
- **Large loading capacity**
- **Monoclonal antibody site-specific conjugation**

## 5. Package Size

20pcs/box; 25pcs/box; 50pcs/box;

## 6. Expiry Date

Expiry Date is one year.

## 7. Reagent Preparation

- 1). 50% acetonitrile (V:V): Add 500mL acetonitrile, then add 500mL purified water.
- 2). PBST: Add 8g NaCl, 0.2g KCl, 0.2g K<sub>2</sub>HPO<sub>4</sub>, 1.2g NaH<sub>2</sub>PO<sub>4</sub>, then mix with 800mL purified water. Later, add 1mL Tween 20 and mix well. After that, adjust PH value to 7.4. At last, get constant volume at 1000mL.
- 3). Eluent: Add 2mL formic acid into 98mL methanol, then mix well.
- 4). Derivative Solution: Add 40mg o-Phthalaldehyde into 1mL methanol. Dilute it with 5mL Borax Solution (0.1 mol/L). Add 50uL 2-mercaptoethanol, then mix well. Put it in a brown bottle, ready to use.



1295 Morningside Avenue, Unit 16-18  
Scarborough, ON M1B 4Z4 Canada  
Phone: 416-261-4865 Fax: 416-261-7879  
[www.scigiene.com](http://www.scigiene.com)

## 8. Sample Preparation and Purification

- 1). Add 5g pulverized sample, then add 25mL 50% acetonitrile; after that, mix them in homogenizer for two mins or vibrate for thirty mins and extract at last.
- 2). Centrifuge at 5000rpm for five mins or filter through rapid qualitative filter paper. Later, take 5mL filtrate, and then add 20mL water or PBST to dilute and mix well.
- 3). Filter by glass microfiber filter paper, then collect filtrate.
- 4). Add 5mL filtrate.

### Note:

1. For sample extracting solution (pH<6 or pH>8), it is necessary to adjust pH value to neutral.
2. For samples that are difficult to filter because of turbidity, centrifuge samples for separation.

## 9. Notes

- 1) Fumonisin Total is harmful to human, so please wear gloves while operation. All glassware exposed to standard/sample should be soaked overnight with 5% sodium hypochlorite solution.
- 2) Do not use expired IAC.
- 3) IAC should be stored at 2~8°C. Do not freeze.
- 4) Equilibrate IAC at room temperature (25°C) for half an hour before use.
- 5) If Fumonisin Total content in sample is higher than column capacity, please decrease sample loading volume accordingly.
- 6) Adjust sample amount and extracting solution volume in proportion according to actual situation. It is recommended to take 5g sample in minimum.
- 7) Do not leave the IAC in a dry state for a long time during the purification process.

## 10. IAC Purification

- 1) Take out IAC, then pierce its upward plug with 10mL injector.
- 2) Fix IAC on Pump Flow Operation Rack or Solid Phase Extraction Apparatus.
- 3) Add 5mL treated sample filtrate into injector, then take off IAC downward plug.
- 4) Adjust flow rate to 1-1.5mL/min.
- 5) After draining the liquid, wash it with 15mL water or PBST at flow rate of 1-1.5mL/min until 2~3 mL air passes through the column to ensure that there is no residual liquid in the column.
- 6) Elute with 1mL eluent (#3 in Reagent Preparation) at flow rate of 1mL/min for three times, and use sample bottle/glass tube to collect the eluent each time. After that, blow dry it with nitrogen at 55°C until it is dry.
- 7) Add 0.5mL 50% acetonitrile to dissolve residue.
- 8) Add 100uL treated solution, then add 100uL Derivative Solution, mix them with Vortex Mixer for thirty seconds. Analyze it in two mins.

## 11. HPLC Instrument Measurement Condition

Chromatographic Column: C18, 5μm, 4.6 mm×250 mm

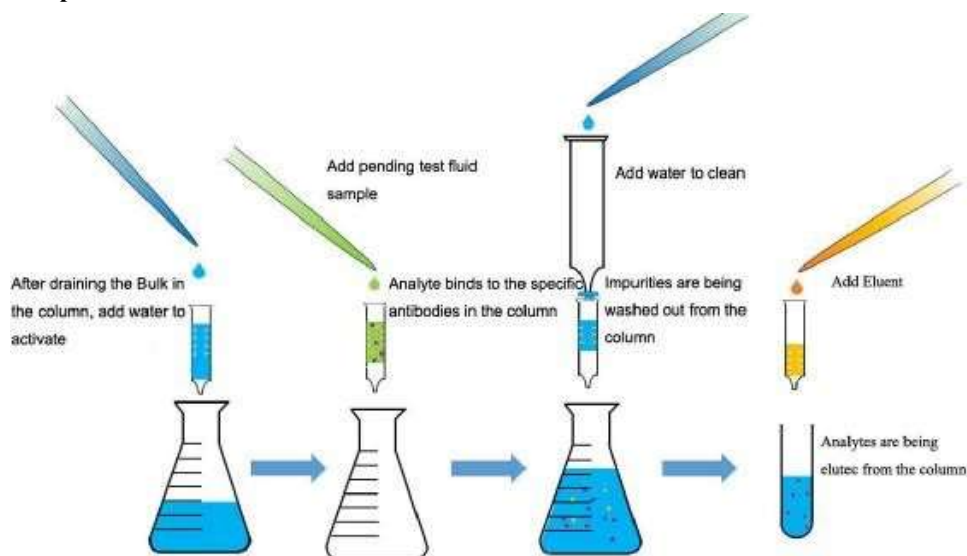
Mobile Phase A: Ammonium Formate-Formic Acid Water Solution

Mobile Phase B: Methanol



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## Sample Dilution Times: 5



**IAC Operation Diagram**

Elute Gradiently:

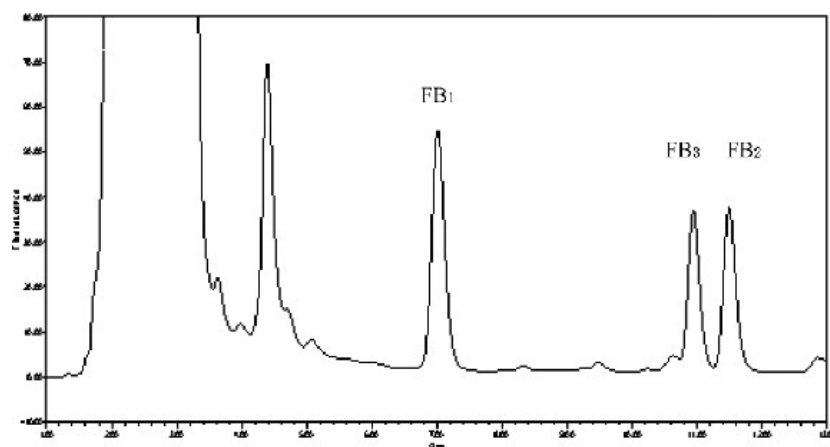
Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	45	55
2	45	55
9	30	70
14	10	90
14.5	10	90
15	45	55
22	45	55

Flow Rate: 1.0mL/min

Detection Wavelength: Excitation Wavelength 335nm, Emission Wavelength 440nm

Sample Loading Quantity: 50µL

Column Temperature: 40°C



**Fumonisin Total Standard Liquid Chromatogram**



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