NEOVENTURES BIOTECHNOLOGY INC.

Afla-Sense® UltraFast M1 Columns for Liquid and Powder whole milk 50ppt-1ppb
Product # 20535

Intended Use
The AFLA-Sense® Ultrafast Column is s purification column for the quantification of aflatoxin M1 in liquid and powder milk. It is used as a clean-up step for analysis with HPLC-FD with and without post-column derivatization.

Aflatoxin

Assay Principles

The AFLA-Sense® UltraFast M1 Column is a DNA-based affinity column. Aflatoxin M1 is sampled from powder or liquid milk. The diluted extracts are purified through the Afla-Sense® UltraFast M1 Column. The amount of total aflatoxins present in a sample can be determined by analyzing the elutant using HPLC-FD. Defatting of the milk is not required to obtain results meeting regulatory limits.

Precautions

1. Adhere to protocols exactly stated. Alteration of the protocol may give inaccurate results.
2. The Afla-Sense® Buffer B contains Tris which is an irritant. Avoid contact with skin or eyes.
3. Consider all materials, containers and devices that are exposed to the sample to be contaminated with toxin. Wear protective gloves and safety glasses when using the kit.
4. Dispose of all materials, containers and devices appropriately after use.

Procedure
Sampling:

Liquid Whole Milk:

1. Dilute 1.5mL of liquid milk with 1.5mL of 2x Buffer B (recipe below).
2. Sample is now ready for purification.

Whole Milk Powder

1. Dilute 5 gram of milk powder with Buffer B heated to 30-40 degrees Celcius to bring the total volume to 50mL.
2. Blend/mix vigorously for 1 minute.
3. Dilute milk solution 1/3-1mL of milk with 2mL of 1.5x Buffer B.
4. Sample is now ready for purification.

Purification:

1. Remove columns from the fridge and equilibrate to room temperature for 5 minutes.
2. Remove the lid and the stopper from the column and allow the storage buffer inside the column to pass through. Apply air pressure using a pipette to the top of the column to speed up the flow of the storage buffer. Do not allow the column to dry out. If the column is allowed to flow by gravity, it will not dry out.
3. Wash the columns with 1mL of Buffer B.
4. Load 2mL of prepared milk solution through the column and allow the sample to flow through by gravity or with air pressure at a maximum of 1drop/sec. If the sample does not flow through immediately, apply slight pressure to the top of the column to start the flow of solution. Do not allow the column to dry out.
5. Wash the column with 700µL of Buffer B. Allow the sample to flow through by gravity or with air pressure at a maximum of 1 drop/sec.
6. Apply air pressure to the column using a 1 mL pipette with a pipette tip to push out all solution in the column and in the resin bed. Blot to remove any residual liquid on the tip and outside of the column.

7. Elute with 500µL of Buffer N 1mM NaOAC pH 3.7, into a fresh 1.5 ml microfuge tube. Allow the elution to flow through by gravity or with pressure at a maximum of 1drop/sec. Apply air pressure to the column using a 1 ml pipette with a pipette tip to push out all the solution in the column and in the resin bed. Inject directly into the HPLC. Elution may also be done in 100%methanol. For highest sensitivity (below 50ppt), dry down the methanol sample in a speed vac and re-suspend in 5-200uL of mobile phase. For rapid screening, straight injections may be performed.
8. The sample is now ready for detection using the HPLC-FD with or without post-column derivatization. Derivatization is only required for higher sensitivity.

Recipes

Buffer B (Binding)	**Buffer N**
10mM Tris pH 7.5	1mM Sodium acetate
120mM NaCl	Adjust pH with acetic acid to 3.7
5mM KCl	
5mM MgCl	

For 2x Buffer B, multiply the components in the recipe by 2x. For 1.5x buffer B, multiply the components in the recipe by 1.5x.

Buffer B and N are available for purchase from NeoVentures.

Preparation of standards for HPLC detection:

The concentrations provided below are adjusted for the extraction and the column purification. They represent the ppb level of mycotoxin present in the original sample.

Liquid Milk: 1ppb or 1ug/L is equivalent to 6.1nM or 2ug/L for HPLC injection.

Powder Milk: 1ppb or 1ug/L is equivalent to 406pM or 133ng/L for HPLC injection for determination of aflatoxin M1 in the actual powder. To determine the liquid milk equivalent, 100 gram of milk is equivalent to 1L of reconstituted milk powder (1gram for 10mL volume of water unless otherwise stated by the manufacturer, do not alter the procedure if the manufacturer dilution is different. The final toxin concentration value in the sample may be adjusted if a liquid milk concentration is required for that specific powder sample). If the powder milk contains 1ug/kg of aflatoxin m1, the liquid milk made from the powder will contain 10x less concentration of aflatoxin m1. (100ng/L) due to the dilution of milk powder in water.

Prepare the standards in the HPLC mobile phase using certified or known concentrations of Aflatoxin M1. Aflatoxin M1 may be purchased from many suppliers in certified concentrations or in powder form. If using the powder form of Aflatoxin is used to prepare the standards, ensure the final concentration of the powder suspension is accurate by either comparing the sample to a certified standard or calculating the sample concentration using extinction coefficients and absorbance readings. When preparing the standards from a concentrated aflatoxin solution, ensure no less than 10µL is pipetted at a time to reduce error.

HPLC Conditions:

HPLC condition 1

Column: reverse phase C18 (Waters Nova pak C18, 3.9mm X 150mm, 4µm column part #WAT086344, Whatman Partisphere RTF C18, 4.6 X 150mm or Merck C18 column, 5mm X 12.5cm, 5µm).
Mobile phase: water:acetonitrile:methanol (68:24:8) isocratic degassed.
Flow rate: 1 mL/min.
Column temperature: 25 °C
Fluorescence detector: excitation 360 nm, emission 440 nm.
Post column:

Reagent: pyridinium hydrobromide perbromide 50mg/L. Solution can be used for up to 4 days if stored in the dark at room temperature.
Apparatus: Second LC pulseless pump, zero-dead volume T-piece, reaction tubing minimum 45 cm _ 0.5 mmid PTFE
Flow rate at 0.3mL/min. Note: This is only necessary for below 20ppt detection. Above 20ppt detection can be achieved using methanol dry down method.

HPLC condition 2

Column: Agilent Zorbax XBD C18 4.6x150mm, 5um
Mobile phase: Water/MeOH/Acetonitrile (68:24:8)isocratic degassed
Fluorescence detector: Excitation 360, Emission 440
Column temperature: 25°C
Injection volume: 50-200uL

Other HPLC protocols may be equally suitable for use.

Materials required but not provided:

- Buffer B
- Buffer N
- Single channel pipettes capable of pippetting 500µL, 700µL, 1mL volumes with tips
- 100% methanol
- Heating block, microwave, or water bath capable of heating water between 30-40 degrees.

Troubleshooting:

Always ensure HPLC quality water or Millipore filtered deionized water is being used. If Millipore filtered deionized water is being used, verify that the water purification system is maintained appropriately. Also ensure the pH meter being used is calibrated and is functioning properly.

Problem: Low recovery of aflatoxin in sample.

Reason: pH of diluted extraction solution is outside of the required range. Aflatoxin will not bind efficiently to the column when the pH is below 6.5 or above 8.
Solution: Adjust the pH of the extract prior to loading into the column using 1M NaOH dropwise.

Reason: pH of the elution buffer 1mM NaOAC is too high. Aflatoxin will only be eluted from the column completely at a pH below 4.
Solution: Adjust the pH of the elution buffer to 3.7.

Reason: The sample was pushed through the column at a rate of greater than 1drop/second. The aflatoxin cannot bind efficiently to the column when a flow rate of more than 1 drop/second is used.
Solution: Only allow the sample to flow by gravity or at a rate of no more than 1 drop/second.

If problems persists, contact NeoVentures Biotechnology Inc.

Warranty

The user assumes all risk in using NeoVentures Biotechnology Inc. products and services. NeoVentures Biotechnology Inc. will, at its option, repair or replace any product, components, or repeat services specific warranty periods or expiration dates and which our examination shall disclose to our satisfaction to be defective in such. This warranty is expressly in lieu of all other warranties, expressed or implied, as to description, quality, merchantability, fitness for any particular purpose, productiveness, or any other remedies, warranties, guarantees or liabilities, expressed or implied, arising by law or otherwise, and it shall have no liability for any lost profits or damage, direct, indirect or otherwise, to person or property, in connection with the use of any of its products or services. This warranty shall not be extended or varied except by written instrument signed by an authorized representative of NeoVentures Biotechnology Inc.

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