NeoVentures Biotechnology Inc.

Afla-Sense® UltraFast Columns 1-200ppb
Reactivity B1 100%:B2 100%: G1 50%: G2 10%

Product # 20530

Intended Use
The AFLA-Sense® Ultrafast Column is s purification column used in combination with the AFLA-Sense® Detection Solution for the quantification of aflatoxins in corn and peanuts. It can also be use as a clean-up step for analysis with HPLC-FD with post-column derivatization.

Aflatoxin

Assay Principles

The AFLA-Sense® UltraFast Column is a DNA-based affinity column. Total aflatoxin is extracted from a ground sample with 80%methanol. The diluted extracts are purified through the Afla-Sense® UltraFast Column. The amount of total aflatoxins present in a sample can be determined either by analyzing the elutant by HPLC-F, or through the use of the AFLA-Sense® System. In the Afla-Sense® System, the elutant is mixed with an enhancement solution and the sample is placed in a microplate well and read in a fluorometer at an excitation of 370 nm and an emission of 440nm. The intensity of the fluorescence signal is directly proportional to the concentration of total aflatoxin in the sample. The relative fluorescence units (RFUs) are compared to the RFUs of the standards and an interpretive result is determined.

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

Extraction

1. Dilute 10 grams of homogeneous ground corn or peanut sample with 40mL of 80% methanol.
2. Blend/mix vigorously for 1 minute.
3. Allow the sample to settle by gravity (about 1 minute) until 2 separate layers are visible. Remove the solution from the liquid phase, the layer above the corn.
4. Dilute the solution 1/5th with Afla-Sense® Buffer B (see recipe below) and mix well. A minimum of 4 mL of diluted filtrate is required for one purification column as a small volume is lost during the next step.
5. Filter through glass wool filter paper (Whatman GF/A or equivalent) and collect filtrate. Filtrate must be clear to proceed. If filtrate is not clear, check filter for any tears that would cause the sample to not be filtered properly. Only proceed if filtrate is clear.
6. Check the pH of the extract using pH paper. pH range must be between 6.5 and 8 with pH 7.5 being optimal. If the pH is below 6.5, adjust the pH to 7.5 by adding 1M NaOH dropwise.
7. Corn extract sample is now ready for purification using the Afla-Sense® UltraFast Columns.

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 3mL of prepared extract 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 (recipe below). 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. For use with the AflaSense® Detection System, 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. For use with HPLC-FD, elute with 500uL of Buffer N 1mM NaOAC pH 3.7 into a fresh 1.5mL tube and inject directly. Elution may also be done in 100%methanol. For highest sensitivity, 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 AflaSense® Detection system or HPLC-FD with 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	

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.

50ppb or 50µg/L is equivalent to 48nM or 15ug/L aflatoxin B1 and 47.7nM or 15ug/L aflatoxin B2 for HPLC injection. Note that the fluorescence of aflatoxin B2 is much stronger than for aflatoxin B1, a smaller concentration may be injected to acquire a standard curve.

Prepare the standards in the HPLC mobile phase using certified or known concentrations of Aflatoxin b1 and B2. Aflatoxin B1 and B2 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: methanol:water (45:55) isocratic degassed.
Flow rate: 0.8 mL/min.
Fluorescence detector: excitation 360 nm, emission 440 nm.
Post column:

Post column iodine: 0.05% iodine solution
(0.5g Iodine, 100mL methanol, 900mL purified water) Dissolve iodine in methanol, stirring until completely dissolved. Add water. Filter solution through 0.45µM nylon filter. This solution can be used for 2weeks.
Flow rate: 0.2 mL/min.
Reaction temperature: 70°C (FIAtron FH-40[Eppendorf] heater & FIAtron [Eppendorf]TC-50 controller).
Reaction time: ~1 minute.

HPLC condition 2

Column: Hypersil ODS 100*2.1mm, 3µm

Mobile phase: Water/MeOH/Acetonitrile 63/26/11

Fluorescence detector: Excitation 365nm, Emission 445or460

Column temperature: 25°C

Injection volume: 5-20uL

Materials required but not provided:

- Buffer B
- Buffer N
- Single channel pipettes capable of pippetting 500µL, 700µL, 1mL volumes with tips
- 100% and 80%methanol

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: Extraction solvent must be 80%methanol. Methanol will evaporate over time. Extraction of aflatoxin from the sample is only efficient at 80% methanol.

Solution: Use freshly made 80%methanol.

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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