AflaOchra™ Immunoaffinity Columns

Item No. 10006167

For the purification of aflatoxins B1, B2, G1, G2 and Ochratoxin A.

PRODUCT DESCRIPTION

AflaOchraStar™ - Immunoaffinity Columns are designed for rapid and simple purification of Aflatoxin B1, G1, B2, G2 and Ochratoxin A from extracts of feed and foodstuffs. AflaOchraStar™ - Immunoaffinity Columns are reliable and user friendly. They contain monoclonal antibodies against aflatoxins and ochratoxins. Aflatoxins[1] are metabolic products of the mold species *Aspergillus flavus* and *Aspergillus parasiticus*. They are highly carcinogenic. Ochratoxins[1] are metabolic products of the molds *Aspergillus* and *Penicillium* and act nephrotoxic.

Maximum levels set by European Commission/2006 are met for all commodity samples. The IAC protocol can be used for all commodities.

INTENDED USE

AflaOchraStar™ - Immunoaffinity Columns are suitable for use in a wide range of commodities including corn, wheat, animal feed and spices; to list a few.

For this process, a representative subsample[2] is extracted with a mixture of solvent. The extract is filtered and then diluted with a buffer. The diluted extract is applied to the AflaOchraStar™ - Immunoaffinity Column, aflatoxins and ochratoxins are bound and can be eluted, after a short rinsing step.

This eluant is suitable for determining the concentration of aflatoxins and ochratoxins directly by HPLC or LC-MS/MS.

WARNINGS AND PRECAUTIONS

1. Mycotoxins are highly toxic substances! Please take care and use protective measures[3]!
2. Please decontaminate any equipment used with 4% solution of sodium hypochlorite.
3. Do not use the AflaOchraStar™ - Immunoaffinity Column after the expiration date indicated on the label.
4. AflaOchraStar™ - Immunoaffinity Columns are designed for single use only.
5. AflaOchraStar™ - Immunoaffinity Columns contain sodium azide.

RECOMMENDED SOLVENTS AND BUFFERS

PBS 10 mM - Buffer (pH 7.4)

Extraction solution:

- 80/20 (v/v) methanol/water
- 84/16 (v/v) acetonitrile/water
- 60/40 (v/v) acetonitrile/water

Methanol for elution, HPLC-grade

Acetic acid (100 %)

All solvents and buffers should be at **room temperature (+20 – 28 °C).** We recommend the use of **Biopure™ mycotoxin standards.**

BIBLIOGRAPHY

[1] CAST (Council for Agricultural Science and Technology) -report, Mycotoxins Risks in Plant, Animal, and Human Systems, Jan. 2003

[2] EC-Directive EC/401/2006, February 23rd 2006, EC-Directive EC/178/2010, March 2nd 2010, laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs.

[3] Tauchmann, F., et al; Alimenta 11, 85 (1972): protective measures

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PROCEDURE

1) Extraction

Grind a representative sample of the commodity. Various mixtures of solvents can be used for the extraction. A mixture of 80/20 (v/v) methanol/water provides good recovery rates. Mixtures of 84/16 (v/v) acetonitrile/water or 60/40 (v/v) acetonitrile/water can be used as well.

For solid samples:

- Weigh out **25 g of sample** into a 250 mL Erlenmeyer flask or whirl-pak bag.
- Add **100 mL of extraction solution** and stopper flask or close bag.
- Shake for 1 hour on a gyratory shaker (250 rpm).
- Using a funnel, filter extract into a sample container through qualitative filter paper.

For liquid samples:

- Samples can be diluted directly with PBS or mixed with solvent to obtain the appropriate ratios of the reagent.
- Any solvent content in the sample must be considered in the calculation of the dilution, or the solvent in the sample can be removed by evaporation if necessary.

2) Dilution of the Extracts

Dilute the extract with PBS until the content of methanol is not higher than 20 % (v/v), the content of acetonitrile should not exceed 10 % (v/v).

E.g. **4 ml extract** (methanol/water 80/20 (v/v)) with **12 ml total PBS (10 mM)**.

Please note: the **pH of the extract should be 6-8**. If necessary, neutralize with NaOH - solution.

3) Sample Application

Place the AflaOchraStar ™ Immunaffinity Column on a closed Luer stopcock. Place a storage vessel with adapter on the AflaOchraStar ™ Immunoaffinity Column and apply the diluted extract. Open the stopcock, the columns usually drip automatically (flow rate 1 - 3 ml / min). The column and the extract must be at room temperature. It is not necessary to rinse the column before adding the extract.

4) Rinse

After the diluted extract has completely passed through, the column must be rinsed. The AflaOchraStar™ - Immunoaffinity Column should be rinsed with **2 x 10 mL PBS (10 mM)**. Use the first portion of the rinse solution to rinse the container. Apply the second portion directly to the AflaOchraStar™ - Immunoaffinity Column.

Any remaining liquid should be removed from the column by applying slight pressure on top of the column or by applying vacuum at the bottom. The column must not dry out during this step.

5) Elution

Remove the syringe barrel from the AflaOchraStar™ - Immunoaffinity Column and place a suitable vial under the column for the collection of the eluant (e.g. HPLC-Vial). Only use HPLC grade methanol for the elution of toxins. Elute in small portions with **1.5 – 3 mL MeOH/HAc (98/2), for e.g. 3 x 0.5 mL**. The methanol/acetic acid should be left on the column for a few seconds before starting elution to allow intensive contact with the gel.

After applying the last portion of methanol/HAc, all remaining liquid has to be removed from the column by applying slight pressure on top of the column or by applying vacuum on the bottom. The eluant can be directly analyzed by HPLC or LC-MS/MS. In case of low-level contamination, the eluant can be dried down (for example with the Romer Evap®-System) and the residue can be dissolved in a small portion of mobile phase (use silanized tubes).

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