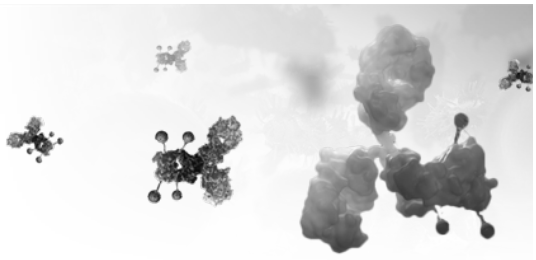

Ochratoxin Immunoaffinity Column (CDM040401)

This product is for research use only and is not intended for diagnostic use.

PRODUCT INFORMATION

Species Reactivity	Ochratoxin A (OTA): 100% Ochratoxin B (OTB): 103% Recovery rate of OTA and OTB divided by recovery rate of OTA if a total of 1.5µg Ochratoxins (with molar ratio of 1:1) is analyzed per column. Please notice that this quantity is around half of the capacity limit of column where binding sites of column are limited. Thus, for analysis within the working range of the column, cross reactivities against OTA and OTB are practically the same in magnitude.
Size	10 Columns
Detection Limit	0.04 –200ng Ochratoxin per IAC
Zero Contamination of Column	<0.04ng (det. lim. of method)
Assay Procedure	1.Extraction (Literature method given): Assuming that 25g sample are extracted by a total of 100ml methanol/water (80/20 v/v). If organic solvent proportion is varied the dilution of extract with PBS should be adapted accordingly in the enrichment step. On the other hand, if proportion of sample quantity and volume of extraction solvent is altered, calculation of gram equivalents must be corrected. Grains could be prepared by the literature method of Olsson et al. For milk as an example of a problematic matrix, method of Zimmerli et al. could be applied. 2.Enrichment Step IAC: 4ml extract (see above, contains the quantity of ochratoxins of 1g sample) are diluted with 16ml 50mM PBS (pH=7.4) and then applied in a reservoir on top of the column. To maintain full performance of the column, please make sure that proportion of dilution buffer in the solution on top of the column is not too small. A proportion of 16% methanol, resulting in this example enrichment, does not affect column performance. Caution! The proportion of organic solvent of PBS diluted extract, which is applied on the

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	column, should not exceed 20% methanol and 10% acetonitrile. If organic solvent proportion lies above these limits, recovery rates are diminished. Increase of diluted extract volume by diluting extract with additional PBS, on the other hand, has almost no consequences to column performance. If samples are to be prepared simultaneously, manifold of J.T. Baker for 12 samples has proven of value. Rate of flow through the affinity gel is 1 to 3 ml/min. In case of problematic matrices rate of flow should lie below 2ml/min. Caution! Be aware that no big air bubbles in the gel or between gel and the lock outlet of column. This can prevent a permanent flow or necessary exchange of matter. Depending on application and on expected contents, larger or smaller extract aliquots can be applied. In such cases the sample calculation (see below) must be adapted. 3.Wash: After whole sample has passed through the gel the latter is washed with 5ml of 10mM PBS/Methanol (90/10 v/v). Remaining liquids in the gel are removed by applying either pressure from top of the column or pressure from bottom. 4.Elution: Sample reservoir on top of the column is removed and a appropriate vial is placed below the affinity column. The bound toxins are eluted by using a total of 1.5ml of methanol/acetic acid (98/2 v/v) as elution solvent. The elution process is performed in two steps to ensure complete release of analytes. First, a volume of 0.5ml elution solvent is applied. After that volume has passed through column half a minute is waited before the second portion of 1ml of elutions solvent is eluted through the column. Remaining solvent solutions should be eluted by application of slight under- or overpressure. All methanolic-acetic acid fractions are unified to give the column eluate. The column eluate may be injected into the HPLC directly or in case concentrations are low it may be concentrated by evaporation, e.g. using VLM evaporator at 50°C under a permanent stream of nitrogen. There is no danger of loss of analytes even though the elute is evaporated to complete dryness. The residue then is redissolved in HPLC solvent and an aliquot is finally injected into the system.
Recovery	>85%±5%. Recovery rates are confined to solvent content of diluted extract below 20% methanol or 10% acetonitrile (see details under Enrichment Step).
Analytical Method	HPLC: Shimadzu Column: Trentec Reprosil-Pur RP C18 120 ODS3 5µm; 125x3,0mm with guard column Mobile Phase A: acetonitrile / deion. water (70/30, v/v) Mobile Phase B: 0.03 M sodium acetate (pH=4,0) / acetonitrile (65/35 v/v) Gradient: 0,01 min B 100 %; 10 min B 100 %; 11 min B 0 %; 13 min B 0 %; 15 min B 100 %; Flow Rate: 0.5ml/min Time of Analysis: 25min Injector Volume: 100µl Fluorescence-Detection: λEX [nm]: 333nm; λEX EM [nm]: 460nm Temperature: Machine and eluents are at room temperature Eluents are degassed with helium gas.

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