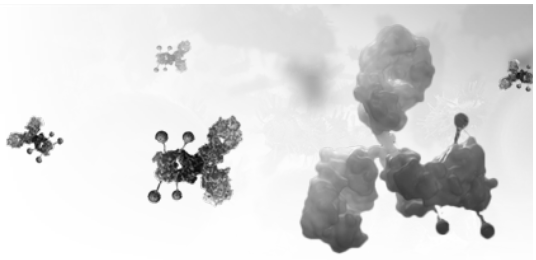

Deoxynivalenol Immunoaffinity Column (CDM040407)

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

PRODUCT INFORMATION

Species Reactivity	DON: 100% Nivalenol (NIV): 31% 15-Acetyl-DON: 33% 3-Acetyl-DON: <1%
Size	10 Columns
Detection Limit	5– 500ng DON per IAC
Zero Contamination of Column	<5ng (LOD of HPLC-UV method)
Assay Procedure	Extraction (Literature method given): Assuming that 50g ground corn sample are extracted by a total of 200ml water, as reported by Cahill et al. If a organic solvent – water mixture is applied instead, 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. Enrichment Step IAC: An aliquot of 1ml extract (see above, contains the quantity of DON of 0.25g sample) are diluted with 9ml 50mM PBS (pH=7.4) to ensure pH of medium is neutral and then applied in a reservoir on top of the column. In case organic solvent – water mixtures are applied as extraction solvents, to maintain full performance of the column, please take care that proportion of dilution buffer in the solution on top of the column is not to small, that means: The proportion of organic solvent of PBS diluted extract, which is applied on the column, should not exceed 15% methanol or 15% acetonitrile. If organic solvent proportion lies above these limits, recovery rates are diminished. Increase of

45-1 Ramsey Road, Shirley, NY 11967, USA
Email: info@creative-diagnostics.com
Tel: 1-631-624-4882 Fax: 1-631-938-8221

	diluted extract volume by diluting extract with additional PBS which then is applied on top of the column, 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 are neither in the gel nor between gel and luer lock outlet of column which 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. Wash: After whole sample has passed through the gel the latter is washed with 20ml of deionized water. Remaining liquids in the gel are removed by applying either pressure from top of the column or underpressure from bottom. Elution: Sample reservoir on top of the column is removed and an appropriate vial is placed below the affinity column. The bound toxins are eluted by using a total of 2ml of methanol as elution solvent. The elution process is performed in two steps to ensure complete release of analytes. First, a volume of 1ml 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 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. Caution: As long as evaporation process is performed moderately, it is not necessary to add a keeper. If that is not the case, it is recommended to use a keeper, e.g. 100µl deionized water or PBS. The residue then is redissolved in HPLC solvent (e.g. 0.5ml) and an aliquot is finally injected into the system.
Recovery	>85%
Analytical Method	HPLC: Shimadzu; Column: Trentec Reprosil-Pur RP C18 120 ODS3 5µm; 125x3,0mm with guard column; Mobile Phase A: methanol / deion. water (85/15, v/v); Mobile Phase B: methanol / deion. water (10/90, v/v); Gradient: 0.01 min B 100 %; 13 min B 100 %; 15 min B 50 %; 16 min B 50 %; 18 min B 100 %; Flow Rate: 0.5ml/min; Time of Analysis: 40min; Injector Volume: 100µl; Detection: UV-Absorbance ABS [nm]: 250nm. Temperature: Machine and eluents are at room temperature. Eluents are degassed with helium gas.

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