QUALICHEK™ Total Ochratoxin Immunoaffinity Column

REF : KBIC1032

Ver 1.0

RUO

Immunoaffinity Column for the use in Quantitative Determination of Ochratoxin.

RUO	**Research Use Only**	**REF**	**log Number**
	e At	**LOT**	**h Code**
	ufactured By		**ogical Risk**
	ry Date		**sult Operating Instructions**

For Research Use Only. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of KRISHGEN BioSystems is strictly prohibited.

REF KBIC1001 Σ 10 columns

KRISHGEN BioSystems | for US / Europe: toll free +1(888)-970-0827 tel: +1(562)-568-5005
for Asia / India: tel: +91(22)-49198700
email: sales@krishgen.com

Introduction:

Ochratoxin is determined in food and feed using Qualichek™ Immunoaffinity Column (Immunoaffinity Chromotography (Qualichek™ IAC)) based on monoclonal antibodies bonded to macroporous pearl cellulose beads in combination with quantification with HPLC (High Performance Liquid Chromotography).

This method of content determination of aflatoxins combines the high selectivity of an immunoaffinity column (IAC) with its potential to concentrate eluate with the additional step of purification by HPLC column. If complex matrices are used for testing, many pretreatment methods of Ochratoxin (OTA A, Ochratoxin B) determination in food and feed will show low sensitivity because of interfering substances.

Intended Use:

The QUALICHEK™ Ochratoxin Immunoaffinity Column is used as an analytical tool for determination of Aflatoxin in grains, feed and food preparations.

Specificity

Ochratoxin A (= OTA).

Form:

Columns filled with gel in phosphate buffered saline (50 mM K-phosphate, pH 7.5, 150 mM NaCl) containing 0.05% sodium azide and 0.01% 2-methyl-4-isothiazolin-3-one embedded within two porous frits.

Gel Bed Volume:

0.3 – 0.5 ml

Column Working Range:

0.04 - 200 ng OTA.
Lower limit only depends on the sensitivity of the detection system (LC-FLD, LC- MS); the given value represents sensitivity of a common LC-FLD system

Recoveries:

85 – 110% of each aflatoxin species within the column working range

Organic Solvent Tolerance:

Up to 20% methanol or 10% acetonitrile in loading solution.

pH Tolerance:

6.5 – 8.5

Flow Rate:

1 - 3 ml/min

Elution:

2 - 3 ml Methanol

Column Capacity:

3000 ng Ochratoxin A

Cross Reactivity:

Ochratoxin A (100%)
Ochratoxin B (103%)

Relative recovery rates when a semi-saturative quantity of aflatoxins (<=900ng) in mixture is applied on the column.

Application:

Clean up of ailment (and feeding stuffs) extracts for reliable identification and accurate quantification of ochratoxins.

Extraction:

1) Weigh 25 gm of sample into high-speed blender jar.
2) Add 100 ml of HPLC Grade Methanol: Distilled Water (80:20, v/v) into the jar.
3) Cover the jar and blend for 1 minute at high speed.
4) Dilute the extract with 125 ml of distilled water.
5) Mix well by swirling
6) Filter approximately 40-50 ml of sample extract through Whatman No. 4 filter paper immediately.

Note:
If the organic solvent proportion is varied, then the dilution of extract with PBS should be adapted accordingly in the enrichment step below, assuming that 25 gm sample is extracted by a total of 100 ml methanol/water (80/20 v/v).

If the proportion of sample quantity and volume of extraction solvent is altered, the 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.

Enrichment Step:

1) 4 ml extract (see above, contains the quantity of aflatoxins of 1 gm sample) is diluted with 16ml 50mM PBS (pH=7.4) and then applied in a reservoir on top of the Qualichek™ IAC Ochratoxin 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 12% methanol, resulting in this example enrichment, does not affect column performance.

Note: The proportion of organic solvent of PBS diluted extract, which is applied on the 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.

2) Rate of flow through the affinity gel is 1 to 3 ml/min. In case of problematic matrices rate of flow should be below 2ml/min.

Note: Be cautious that there are no big air bubbles in the gel or between gel and the lock outlet of column. This can prevent a permanent flow or the 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:

1) After the entire sample has passed through the gel, wash the gel with 5ml of 10mM PBS/Methanol (90/10 v/v).
2) Remove the remaining liquids in the gel by applying either pressure from top of the column or pressure from bottom.

Elution:

1) Remove the Sample reservoir on top of the Qualichek™ IAC Ochratoxin column.
2) Place an appropriate vial below the affinity column.
3) Elute the bound toxins 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.
4) A volume of 1ml elution solvent is applied. Allow the entire volume to pass through the column.
5) Wait for 30 seconds.
6) Apply additional 1ml of elution solvent through the column. The flow rate of the elution process should not exceed 1 ml/min.
7) Elute the remaining solvent solutions by application of slight under or over pressure. All methanolic fractions are unified to give the column eluate.

HPLC:

The column eluate may be injected into the HPLC directly.

If the contents are low (<2 ng/gm, related to commodity content), it may be concentrated by evaporation (e.g. using a VLM evaporator at 50°C under a permanent stream of nitrogen).

Note: If the sample is required to be concentrated by evaporation; add 100 ul of acetic acid/water (50/50 v/v) as keeper. To avoid loss of analytes, stop the concentration at a small volume of residue, e.g. 50 to 100 ul. The residue then is redissolved in HPLC solvent (e.g. 0.4ml) and an aliquot is finally injected into the system.

HPLC Conditions:

Instrument: Shimadzu HPLC

HPLC: Shimadzu	
Column:	Trentec Reprosil-Pur RP C18 120 ODS3 5 um; 125 x 3,0 mm 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:	0.5 ml/min
Time of Analysis:	25 min
Injector Volume:	100 ul
Fluorescence-Detection:	λ EX [nm]: 333nm; λ EM [nm]: 460nm
Temperature:	Machine and eluents are at room temperature. Eluents are degassed with helium gas.

Qualichek™ IAC Column Characteristics:

A) Working Range and Recovery Rates of Qualichek™ IAC Aflatoxin Column:
Working Range of Column: 0.04 - 200 ng Ochratoxin per IAC
Zero Contamination of Column: <0.04 ng (LOD of HPLC-FLD method)

Guaranteed Recovery Rates(*) within the Working Range:
Ochratoxin: >85% +/-5%

* Recovery rates are confined to solvent content of diluted extract below 20% methanol or 10% acetonitrile (see details under Enrichment Step).

B) Cross Reactivities of Qualichek™ IAC Aflatoxin Column:

Ochratoxin A (100%)
Ochratoxin B (103%)

** Recovery rate of OTA and OTB divided by recovery rate of OTA if a total of 1.5 ug 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.

C) Capacity of Qualichek™ IAC Ochratoxin Column:

Maximum Column Capacity: 3 ug Ochratoxin total

*** An excess of ochratoxin, e.g. 4 ug, in a small volume of 2 ml PBS is incubated with the IAC for 5 minutes; then the IAC is washed with 2 ml PBS and the non-bonded fraction is analyzed. The difference of added analyte and non-bonded analyte equals maximum column capacity.

HPLC Method Characteristics:

Measuring Range: Linear 10 pg - 1000 pg Ochratoxin A (OTA) per injection (R2=0.999) in given HPLC method. Lower Limit of Detection (LOD): 10 pg OTA per injection (signal to noise (S/N) ratio = 3).

Balance of Commodity Contamination and HPLC Measuring Range:

OTA contents in commodities of 0.04 to 4ng/g lie within measuring range of HPLC method. If contents are higher, from 4 - 200 ng/g, the IAC column eluate should be diluted accordingly with HPLC eluent or, alternatively, injector volume should be adjusted.

Only if commodity contents lie above working range of IAC column of 200 ng/gm OTA, the 1 gm equivalent per column of this instruction must be lowered. Thus, the IAC enrichment step and subsequent HPLC analysis should be repeated with a smaller extract volume, e.g. instead of 4 ml extract a volume of 2 ml extract, or even less where applicable, are diluted with 10 ml PBS and applied to the IAC column.

Calculation of Results:

Example Sample Calculation of OTA content. (Calculation of OTB content is analogous)

A) Calculation of Sample Gramm Equivalents per HPLC injection:

$$\frac{\text{25 gm Sample}}{\text{100 ml Extraction Solvent}} \times \frac{\text{4 ml Extract}}{\text{0.4 ml}} \times \text{0.1ml Injector Volume} = \text{0.25 gm Sample Equivalents}$$

B) Calculation of OTA contamination of examined commodity in ng/gm:

$$\frac{\text{\# ng injected OTA}}{\text{Sample Equivalents [gm]}} = \text{ng/gm OTA in e.g. ground nut meal}$$

Storage:

The performance of the immuno affinity column is guaranteed until the expiry date given on the label or certificate if stored at 4°C. Avoid freezing of the product.

Reference:
"Determination of aflatoxins in groundnut meal by high-performance liquid chromatography: a comparison of two methods of derivatisation of aflatoxin B1"
G. Roch, G. Blunden, J. Haig, D.Coker and C. Gay B. J. Biomed Sci 1995, 52, 312-316.

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems shall be to repair or replace the defective Products in the manner and for the period provided above. Krishgen Biosystems shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, and strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

Krishgen Biosystems. 2020

THANK YOU FOR USING KRISHGEN PRODUCT!