QUALICHEK™ Total Aflatoxin Immunoaffinity Column

REF : KBIC1001
Ver 1.0

RUO

Immunoaffinity Column for the use in Quantitative Determination of Total Aflatoxin.

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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10 columns

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Introduction:

Total Aflatoxin 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 aflatoxin (AFG2, AFG1, AFB2 and AFB1) determination in food and feed will show low sensitivity because of interfering substances.

Intended Use:

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

Specificity

Aflatoxins (AFL) (= AFB1, AFB2, AFG1 and AFG2).

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 – 0.5ml

Column Working Range:

0.04 – 200 ng AFL.
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 – 3ml Methanol

Column Capacity:

1800 ng aflatoxins (AFL)

Cross Reactivity:
Aflatoxin B1 (100%)
Aflatoxin B2 (86%)
Aflatoxin G1 (101%)
Aflatoxin G2 (82%)
Aflatoxin M1 (81%)
Relative recovery rates when a semi-saturative quantity of aflatoxins (<=900 ng) in mixture is applied on the column.

Application:

Clean up of ailment (and feed stuff) extracts for reliable identification and accurate quantification of Aflatoxins.

Extraction:

1) Weigh 25 gm of sample and 2 gm of Sodium Chloride into high-speed blender jar.
2) Add 125ml of HPLC Grade Methanol: Distilled Water (60:40, 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 (60/40 v/v).

If the proportion of sample quantity and volume of extraction solvent is altered, the calculation of gram equivalents must be corrected. For groundnut meal as an example of a problematic matrix, literature method of Roch et al.1 maybe be applied.

Enrichment Step:

1) 4 ml extract (see above, contains the quantity of aflatoxins of 1 gm sample) is diluted with 16 ml 50 mM PBS (pH=7.4) and then applied in a reservoir on top of the Qualichek™ IAC Aflatoxin 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 5 ml of 10 mM 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 Aflatoxin column.
2) Place an appropriate vial below the affinity column.
3) Elute the bound toxins by using a total of 2 ml 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

Column:	Trentec Reprosil-Pur RP C18 120 ODS3 5 µm; 125x3,0 mm with guard column
Mobile Phase A:	methanol / deionized water (85/15 v/v)
Mobile Phase B:	methanol / acetonitrile / deionized water (18/18/64 v/v/v)
Gradient:	0.01 min B 100 %; 16 min B 100 %; 17 min B 0 %; 19 min B 0 %; 20 min B 100 %
Flow Rate:	0.5ml/min
Time of Analysis:	30min Injector Volume: 100µl
Post Column Derivatization:	32 ppm pyridinium hydrobromide perbromide in dioxin / deionized water (0.1/99.9, v/v)
Flow Rate:	0.5ml/min; reaction capillary (put between end of column and detector by T-device): PEEK-capillary, 1/16´´ x 0.25 mm ID; length: 40 cm
Fluorescence-Detection:	EX [nm]: 362nm; EM [nm]: 440nm.
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 Aflatoxin total per IAC
Zero Contamination of Column: <0.04 ng (LOD of HPLC-FLD method)

Guaranteed Recovery Rates (*) within the Working Range:
Aflatoxin Total: >90% +/-5%
Aflatoxin B_1 (AFB_1): >95% +/- 5%
Aflatoxin B_2 (AFB_2): >90%% +/-5%
Aflatoxin G_1 (AFG_1): >95%% +/-5%

Aflatoxin G_2 (AFG_2): >90% +/-5%

* Recovery Rates are confined to the complete IAC separation procedure.

B) Cross Reactivities of Qualichek™ IAC Aflatoxin Column:
Aflatoxin B1 (AFB1): 100%
Aflatoxin B2 (AFB2): 86%
Aflatoxin G1 (AFG1): 101%
Aflatoxin G2 (AFG2): 82%

** Recovery rate of AFB2, AFG1, AFG2, divided by recovery rate of AFB1 if a total of 1.8µg Aflatoxin total (with molar ratio of B1, B2, G1, G2, = 4:1:4:1) is analyzed per column. Please note that this quantity is near the capacity limit of column where binding sites of column are limited. For this reason, cross reactivities of AFG1 and AFG2 are much higher at aflatoxin concentrations within the given working range where antibody binding sites are in excess (see above).

C) Capacity of Qualichek™ IAC Aflatoxin Column:
Maximum Column Capacity: 1.8 ug Aflatoxin total

*** is incubated with the IAC for 5 minutes; then the IAC is washed with 2ml PBS and the non-bonded fraction is analyzed. The difference of added analyte and non-bonded analyte equals maximum column capacity.

HPLC Method Characteristics:

Aflatoxin B1
Measuring range is linear from 10 pg to 500 pg Aflatoxin B1 per injection (R2=0.999) in given HPLC method.
Lower limit of detection (LOD) is 2 pg AFB1 per injection (signal to noise (S/N) ratio = 3).

Balance of Commodity Contamination and HPLC Measuring Range:
1) If the stated dilution steps (enrichment stage, Lucia concentration stage) are followed, the content of aflatoxin B1 in the 0.04 to 2 ng/g commodites is within the measurement range of the HPLC method.
2) If the content is higher, from 2 to 200 ng/g, the IAC column elitshould be diluted accordingly with HPLC evade or alternatively, the injector volume should be adjusted.

3) Only if the contents of the products are above the working range of the iac column of 200 ng/g of total aflatoxin, the equivalent of 1 gm per column of this statement must be lowered. Thus, the iac enrichment stage and subsequent HPLC analysis should be repeated with a smaller extract volume, for example instead of the 4ml extract a 2ml extract volume, or even less applicable case, are diluted with 10ml OF PBS and applied to the IAC column.

Performance Characteristics:

Left Aflatoxin G1, Aflatoxin B2; Aflatoxin G2.
Sensitivities [AREA Peak / pg] of aflatoxins AFG1, AFB2 und AFG2 related to that of AFB1 are 37%; 136% and 67%, respectively.
AFG1: Measuring range is linear from 25pg to 500pg Aflatoxin G1 per injection (R2 =0.999).
LOD of detection is 6pg AFG1 per injection (signal to noise (S/N) ratio = 3).
AFB2: Measuring range is linear from 2.5pg to 125pg Aflatoxin B2 per injection (R2 =0.999).
LOD is 1pg AFB2 per injection (signal to noise (S/N) ratio = 3).
AFG2: Measuring range is linear from 2.5pg to 125pg Aflatoxin G2 per injection (R2 =0.999).
LOD is 2pg AFG2 per injection (signal to noise (S/N) ratio = 3).

Because left aflatoxins (AFG1, AFB2 and AFG2) mostly occur in common and are thus analyzed simultaneously with key aflatoxin B1, different levels of commodity contents and established HPLC measuring ranges are balanced by the same manner as stated for aflatoxin B1.

Recoveries of Aflatoxin using Qualicheck™ IAC				
Matrix	**B1**	**B2**	**G1**	**G2**
Almonds	91 %	91 %	90 %	79 %
Chili	116 %	117 %	121 %	92 %
Cinnamon	87 %	85 %	86 %	87 %
Dates	108 %	96 %	116 %	72 %
Dried Distillers Grain	109 %	97 %	90 %	77 %
Figs	115 %	104 %	110 %	74 %
Hazelnuts	98 %	100 %	95 %	83 %
Maize	101 %	98 %	103 %	80 %
Peanut Butter	95 %	98 %	93 %	84 %
Peanuts	114 %	113 %	102 %	91 %
Pistachios	90 %	90 %	100 %	75 %
Rice	103 %	104 %	104 %	89 %

Calculation of Results:

Example Sample Calculation of AFB1 content. (Calculation of AFB2, AFG1 and AFG2 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 Aflatoxin B1 contamination of examined commodity in ng/g:

$$\frac{\text{\# ng injected AFB1}}{\text{Sample Equivalents [g]}} = \text{ng/g AFB1 in e.g. ground nut meal}$$

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.

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