QUALICHEK™ Aflatoxin M1 Immunoaffinity Column

REF : KBIC1003

Ver 1.0

RUO

Immunoaffinity Column for the use in Quantitative Determination of Total Aflatoxin M1.

IVD	**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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REF KBIC1003 Σ 10 columns

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Introduction:

Total Aflatoxin M1 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 Aflatoxin M1 s 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 M1 (AFG2, AFG1, AFB2 and AFB1) determination in food and feed will show low sensitivity because of interfering substances.

Intended Use:

The QUALICHEK™ Total Aflatoxin M1 Immunoaffinity Column is used as an analytical tool for determination of Aflatoxin M1 in grains, feed and food preparations.

Specificity

Aflatoxins M1 (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 - 50 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 Aflatoxins M1 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:

900 ng Aflatoxin M1 s (AFL)

Cross Reactivity:

Aflatoxin B1 (>90%)	Aflatoxin B2 (>90%)
Aflatoxin G1 (>90%)	Aflatoxin G2 (>90%)

Relative recovery rates when a semi-saturative quantity of Aflatoxins M1 (<=900ng) in mixture is applied on the column.

Application:

Clean up of milk samples for reliable identification and accurate quantification of Aflatoxin M1.

Extraction:

1) Centrifuge the mlk sample at 1540 g for 15 minutes.
2) The cream is then separated using e.g. a separation funnel. If separation is incomplete, re-centrifuge.
3) Filter approximately 40-50 ml of sample extract through Whatman No. 4 filter paper immediately.

Note:
The milk sample should be filtered to avoid the fatty particles blocking the immunoaffinity column.

Enrichment Step:

1) 50 ml extract is diluted with 5 ml 10X PBS (= 10fold concentrated 50mM PBS buffer, pH=7.4) and then applied in a reservoir on top of the Qualichek™ IAC Aflatoxin M1 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.

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 50mM PBS.
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 M1 column.
2) Place an appropriate vial below the affinity column.
3) Elute the bound toxins by using a total of 1.5 ml of methanol as elution solvent. The elution process is performed in two steps to ensure complete release of analytes.
4) A volume of 0.5 ml elution solvent is applied. Allow the entire volume to pass through the column.
5) Wait for 30 seconds.
6) Apply additional 1 ml 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 m; 125x3,0mm 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:	100 ul

Post Column Derivatization
(only advisable in case of concomitant aflatoxin B1 analysis): 32 ppm pyridinium hydrobromide perbromide in dioxan/ deionized water (0.1/99.9, v/v)

Flow Rate:	0.2ml/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:	λ EX [nm]: 362nm; λ 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 M1 Column:
Working Range of Column: 0.04 - 200ng Aflatoxin M1 total per IAC
Zero Contamination of Column: <0.04ng (LOD of HPLC-FLD method)

Guaranteed Recovery Rates (*) within the Working Range:
Aflatoxin M1: >85% +/-5%

* Recovery rates are confined to the complete IAC separation procedure. Recovery rates are confined to solvent content of diluted extract below 20% methanol or 10% acetonitrile.

B) Cross Reactivities of Qualichek™ IAC Aflatoxin M1 Column:
Aflatoxin M1 (AFM1): 100%
Aflatoxin B1 (AFB1): 98%
Aflatoxin B2 (AFB2): 99%

Aflatoxin G1 (AFG1): 50%
Aflatoxin G2 (AFG2): 98%

** Ratio of IAC recovery rates if a quantity of 5ng Aflatoxin total per column is analyzed

C) Capacity of Qualichek™ IAC Aflatoxin M1 Column:

Maximum Column Capacity: 1.8 ug Aflatoxin M1 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.

Calculation of Results:

Example Sample Calculation of AFM1 content.

A) Calculation of Sample Gram Equivalents per HPLC injection:

$$\frac{\text{50 gm Sample}}{\text{55 ml Extraction Solvent}} \times \frac{\text{55 ml Extract}}{\text{0.4 ml}} \times \text{0.1ml Injector Volume} = \text{12.5 gm Sample Equivalents}$$

B) Calculation of Aflatoxin M1 contamination of examined commodity in ng/gm:

$$\frac{\text{\# ng injected AFM1}}{\text{Sample Equivalents [gm]}} = \text{ng/gm AFM1 in commodity}$$

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:
"Aflatoxin M1 in milk by immunoaffinity column cleanup with TLC/HPLC deterimination" Luzia Shundo; Myrna Sabino, Brazilian Journal of Microbiology 2006, 37,164-167

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