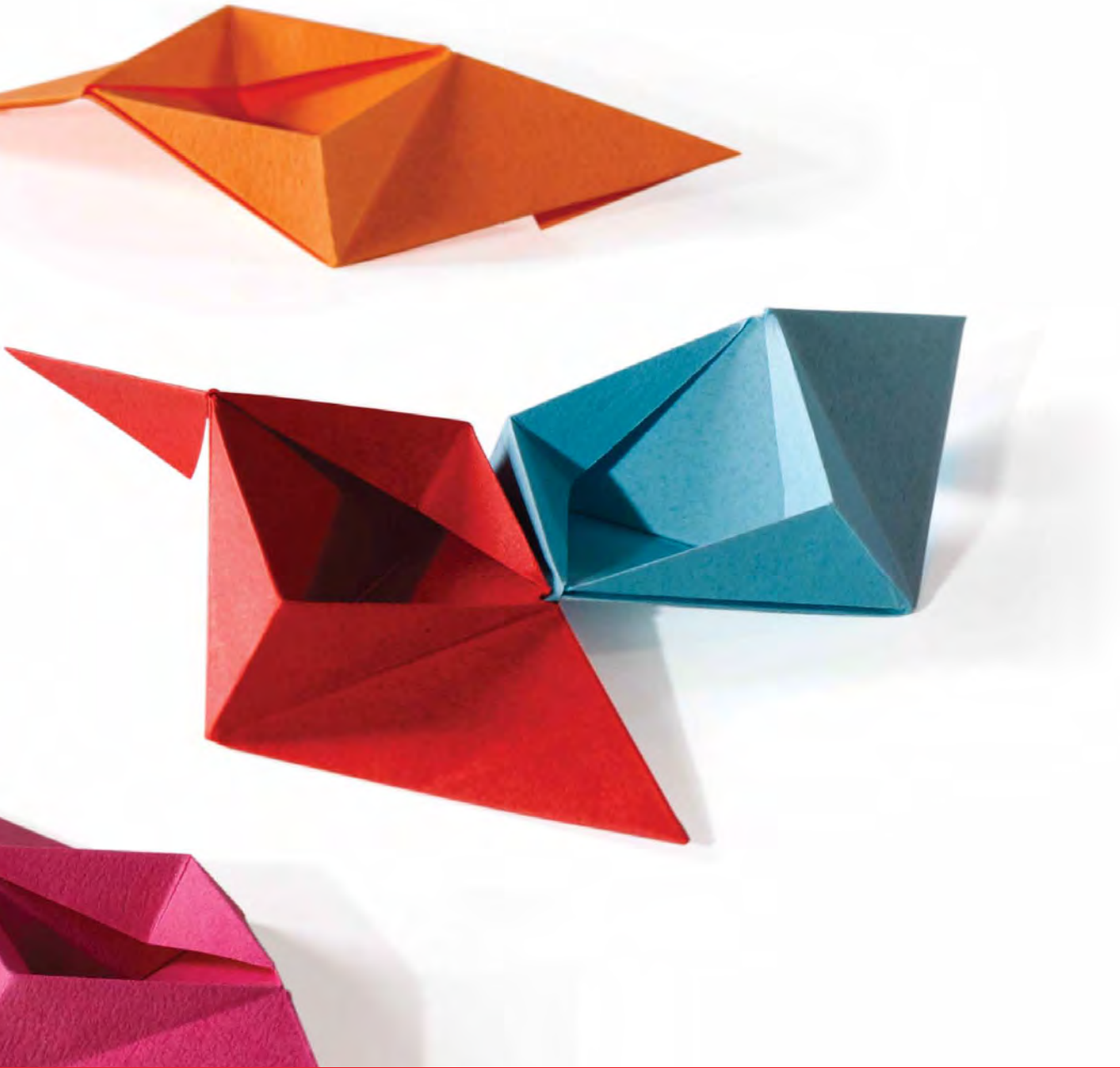
SUPELCO®
Solutions within.™

Supel™ Tox SPE Cartridges

Fast and Simple Cleanup for Mycotoxin Analysis



SIGMA-ALDRICH®

Supel™ Tox SPE Cartridges

Specialty Products for Mycotoxin Analysis

Features and Benefits

- Remove interferences associated with mycotoxin analysis
- Better reproducibility than the current industry standard, immunoaffinity columns
- Time associated with sample preparation is up to ten times less than that of immunoaffinity columns
- Basic and quick methodology requiring little additional method development
- Improved shelf life over immunoaffinity columns due to thermally stable format. No refrigeration required for shipping and storage.

The need for a quick, simplistic sample cleanup approach prior to chromatographic mycotoxin analysis has brought about SPE cartridges that significantly decrease sample prep time, increase reproducibility, and are more user friendly as compared to the industry standard immunoaffinity columns. In addition, the Supel Tox SPE approach requires less equipment and fewer consumables, providing an additional cost savings.

Unlike the multiple step "bind and elute" strategy implemented when using immunoaffinity columns, the Supel Tox AflaZea, DON and Tricho SPE cartridges employ an "interference removal" strategy

which saves time by eliminating wash steps prior to elution of aflatoxin and zearalenone, deoxynivalenol, and tricothecenes (type A and B), respectively. Cartridges removing interferences associated with analysis of fumonisins (B_1 and B_2) and ochratoxin A are also available as a part of our Supel Tox product offering. Refer to the table below for further details.

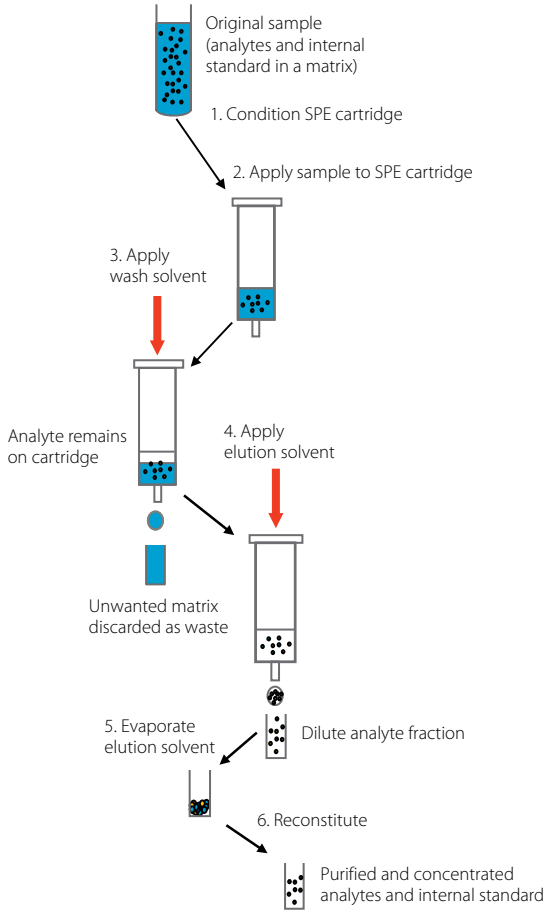
Sample Purification Strategy for the Various Supel Tox SPE Cartridges

SPE Cartridge	Analyte(s)	Matrix	Purification Strategy
Supel Tox AflaZea	Aflatoxin and Zearalenone	Grains, feeds, TMR samples, peanuts, peanut products and aqueous solutions	Interference removal
Supel Tox DON	Deoxynivalenol (DON)	Wheat, flour and corn	Interference removal
Supel Tox Tricho	Trichothecenes (Type A and B)	Grains and complex matrices	Interference removal
Supel Tox TrichoBind	Trichothecenes (Type A and B)	Grains, feeds and other complex matrices	Multifunctional bind and elute
Supel Tox FumoniBind	Fumonisins (B_1 and B_2)	Grains and cereals	Multifunctional bind and elute
Supel Tox OchraBind	Ochratoxin A	Grain and feed samples	Multifunctional bind and elute

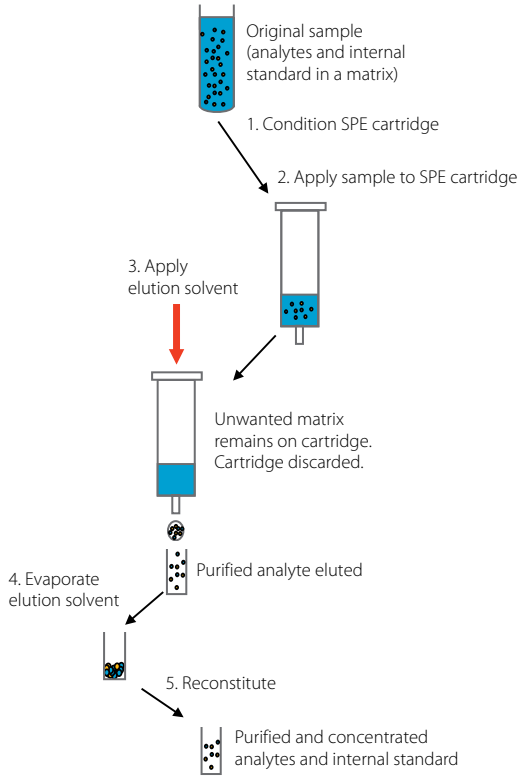
Comparison of "Bind and Elute" Principle to "Interference Removal" Principle

In the "bind and elute" strategy, the analyte of interest becomes bound to the SPE sorbent. The unwanted matrix is removed, and the analyte is ultimately eluted. In "interference removal," the unwanted matrix components remain bound to the sorbent, while the purified analyte is eluted. This strategy reduces the number of purification steps, allowing for time savings and increase in sample throughput. The Supel™ Tox AflaZea, DON and Tricho SPE cartridges employ the "interference removal" strategy.

"Bind and Elute" Principle



"Interference Removal " Principle

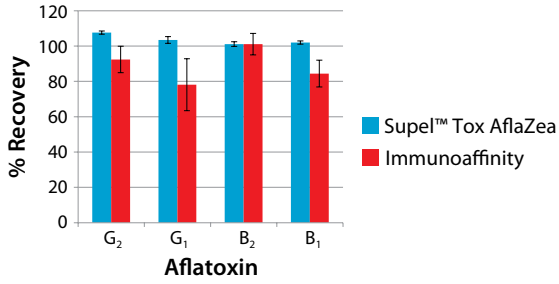


Comparison of Supel™ Tox SPE Cartridges to Immunoaffinity Columns for Matrix Removal Prior to Mycotoxin Analysis

- Ten times faster – ten times sample throughput
- Vast improvement in reproducibility

The following is an experiment comparing the use of the Supel Tox AflaZea SPE cartridge to a leading immunoaffinity column for the analysis of aflatoxins B_1, B_2, G_1 and G_2 in peanut paste. The results illustrate Supel Tox AflaZea SPE cartridges are superior to immunoaffinity columns in terms of process simplicity, time required for sample preparation, and control of variation.

Cleanup of Aflatoxins in Peanut Paste: Immunoaffinity Versus Supel™ Tox (n = 3)



Comparison of Sample Preparation Methods Using Supel Tox AflaZea SPE vs Immunoaffinity for Aflatoxins in Peanut Paste

	Immunoaffinity	Supel Tox AflaZea SPE Cartridge
Sample Prep Time (post-extraction to pre-analysis)	• 60 minutes • 8 samples/day (if processing 1 at a time)	• 6 minutes • 80 samples/day (if processing 1 at a time)
Ease of Use	• Large volumes of liquid • Controlled drop rates • Numerous complicated steps • Additional buffer salts required • Must be refrigerated, brought to room temp before use	• Small volumes of liquid • Vacuum filtration used • Steps few and not complicated • No additional reagents required • Column does not require special storage conditions
Procedure (post-extraction to analysis)	Stage 1 (15 minutes) 1. Configure manifold for waste collection 2. Add 1 mL sample to 17 mL of phosphate buffered saline, vortex 3. Uncap/mount/drain cartridges by gravity 4. Apply reservoirs, load sample onto cartridges Stage 2 (15 minutes) 5. Rinse interferences 6. Reconfigure manifold for sample collection 7. Elute/collect sample Stage 3 (30 minutes) 8. Evaporate sample to dryness 9. Reconstitute sample and vortex 10. Transfer 0.2 mL sample to vial 11. Dilute sample and vortex Analysis	Purify and Transfer (6 minutes) 1. Configure manifold for sample collection 2. Mount cartridges 3. Load 2 mL sample 4. Elute and collect under vacuum 5. Transfer 0.2 mL sample to vial 6. Dilute sample and vortex Analysis

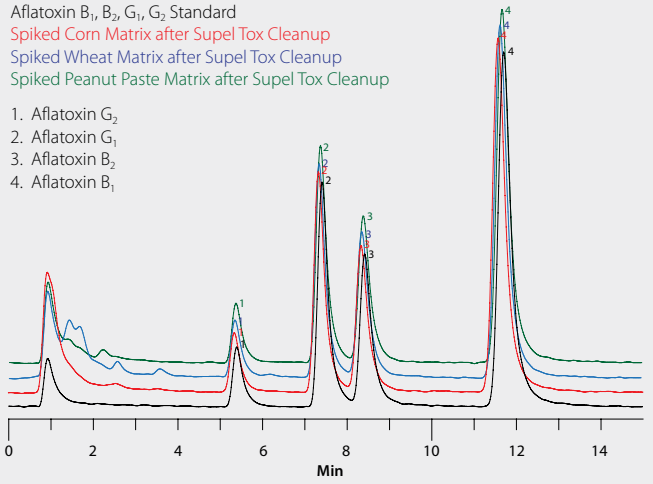
Analysis of Aflatoxin B_1, B_2, G_1 and G_2 in Corn Meal, Wheat Meal and Raw Peanut Paste Matrices

The Supel Tox AflaZea SPE cartridge was used to demonstrate the cleanup of corn meal, wheat meal and raw peanut paste matrices prior to the analysis of Aflatoxin B_1, B_2, G_1 and G_2. In the procedure, corn meal, wheat meal and raw peanut paste extracts were spiked with Aflatoxin Standard Mix (Cat. No. 34036) and run through the Supel Tox AflaZea SPE cartridges. The eluate was collected and diluted for HPLC analysis. Results illustrate Supel Tox AflaZea SPE cartridges provide exceptional cleanup of corn meal, wheat meal and raw peanut paste matrices with high analyte recoveries and low %RSD for aflatoxin analysis.

Analyte Recovery and %RSD of Aflatoxins from Corn, Wheat and Peanut Paste (n = 3)

Aflatoxins in Corn Meal, Wheat Meal and Raw Peanut Paste After Supel Tox Cleanup

column:	Ascentis® Express C18, 10 cm x 2.1 mm I.D., 2.7 µm (53823-U)
mobile phase:	(A) water; (B) acetonitrile; (C) methanol; (700:120:120,A:B:C) with 0.780 g potassium bromide and 230 µL of nitric acid
flow rate:	0.400 mL/min
column temp.:	35 °C
detector:	Fluorescence detector, Excitation: 360 nm, Emission: 440 nm
injection:	40 µL



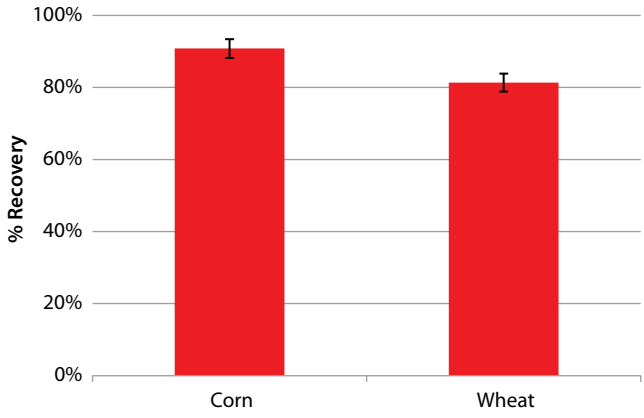
Analysis of Corn Meal and Wheat Meal Matrices for Deoxynivalenol

Deoxynivalenol (DON), also referred to as vomitoxin, is a concern primarily in the livestock industry, as it is known to cause a reduction in food intake in farm animals. DON is a type B trichothecene which may occur in a variety of grains.

Cleanup of corn meal and wheat meal matrices for the analysis of DON was illustrated with the use of Supel™ Tox DON SPE cartridges. The grain extracts were spiked with deoxynivalenol standard (Cat. No. 34124) and applied to the cartridges. The eluate was collected, evaporated, and reconstituted for HPLC analysis. Blank unspiked matrix samples were also run though cartridges and analyzed. For the recovery calculations, the peak area of DON observed in the blanks was subtracted from that of the spiked samples.

The corresponding bar chart and chromatograms illustrate that in addition to effectively removing the unwanted corn and wheat meal matrices, Supel™ Tox DON SPE cartridges also provide high analyte recoveries and reproducibility for the analysis of DON.

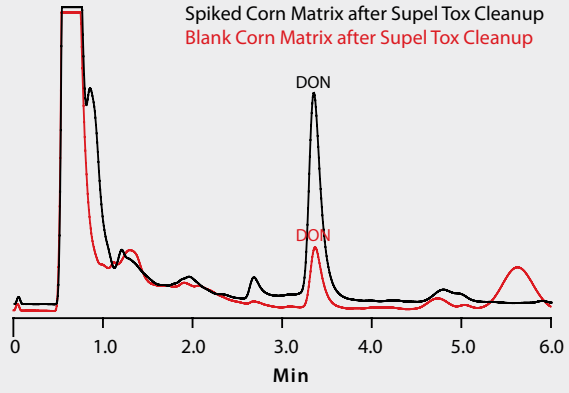
Analyte Recovery and %RSD of Deoxynivalenol (DON) from Corn and Wheat (n = 3)



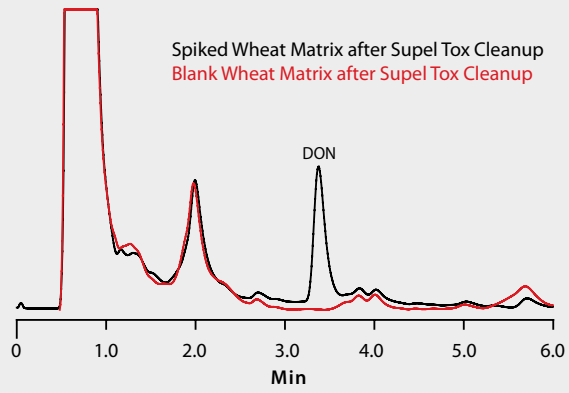
Deoxynivalenol in Corn and Wheat Meal After Supel Tox Cleanup

column:	Ascentis® Express C18, 10 cm x 2.1 mm I.D., 2.7 µm (53823-U)
mobile phase:	(A) water; (B) acetonitrile; (C) methanol; (92:4:4, A:B:C)
flow rate:	0.400 mL/min
column temp.:	35 °C
detector:	UV 220 nm; 360 nm
injection:	40 µL

Corn Meal



Wheat Meal



Supel™ Tox Product Offering

Supel Tox SPE Cartridges

Description	Qty.	Cat. No.
Supel Tox AflaZea SPE Cartridge, 6 mL	30	55314-U
Supel Tox DON SPE Cartridge, 6 mL	30	55316-U
Supel Tox Tricho SPE Cartridge, 6 mL	30	55308-U
Supel Tox TrichoBind SPE Cartridge, LRC	25	55307-U
Supel Tox FumoniBind SPE Cartridge, LRC	25	55315-U
Supel Tox OchraBind SPE Cartridge, LRC	25	55318-U

Featured and Related Products

Description	Qty.	Cat. No.
Ascentis Express HPLC Columns (2.7 µm particles)		
C18, 10 cm x 2.1 mm I.D.	1	53823-U
C18, 15 cm x 2.1 mm I.D.	1	53825-U
RP-Amide, 10 cm x 2.1 mm I.D.	1	53913-U
RP-Amide, 15 cm x 2.1 mm I.D.	1	53914-U
F5, 10 cm x 2.1 mm I.D.	1	53569-U
F5, 15 cm x 2.1 mm I.D.	1	53571-U
Ascentis Express Guard Columns (2.7 µm particles)		
Holder	1	53500-U
C18 Cartridge, 5 mm x 2.1 mm I.D.	3	53501-U
RP-Amide Cartridge, 5 mm x 2.1 mm I.D.	3	53514-U
F5 Cartridge, 5 mm x 2.1 mm I.D.	3	53594-U
Laboratory Equipment		
Coors™ Büchner Funnel, capacity 186 mL	1	Z247324
Individual Filter Adapters, size 3	1	Z254274
Visiprep™ Solid Phase Extraction Manifold, 12-port Model	1	57030-U
Visiprep Solid Phase Extraction Manifold, 24-port Model	1	57250-U
Visiprep DL Solid Phase Extraction Manifold, 12-port Model	1	57044
Visiprep DL Solid Phase Extraction Manifold, 24-port Model	1	57265
Disposable Valve Liners, PTFE	100	57059
Visi™-1 Single SPE Tube Processor	1	57080-U
SPE Tube Adapters for 1, 3, 6 mL tubes	12	57020-U
Mycotoxin Reference Materials (Neats)		
3-Acetyldeoxynivalenol	5 mg	32927
15-Acetyldeoxynivalenol	5 mg	32928
Aflatoxin B_1	5 mg	32754*
Aflatoxin B_2	5 mg	32755
Aflatoxin G_1	5 mg	32756
Aflatoxin G_2	5 mg	32757*
Deoxynivalenol	5 mg	32943
Fumonisin B_1	5 mg	32936
Fusarenon X	5 mg	33438
Ochratoxin A	5 mg	32937
T-2 Toxin	5 mg	33947*
Zearalenone	5 mg	32939
Mycotoxin Single Component Standard Solutions		
3-Acetyldeoxynivalenol, 100 µg/mL in acetonitrile	2 mL	34132
15-Acetyldeoxynivalenol, 100 µg/mL in acetonitrile	2 mL	34133
Aflatoxin B_1, 2 µg/mL in acetonitrile	2 mL	34029
Aflatoxin B_2, 0.5 µg/mL in acetonitrile	2 mL	34034
Aflatoxin G_1, 2 µg/mL in acetonitrile	2 mL	34032
Aflatoxin G_2, 0.5 µg/mL in acetonitrile	2 mL	34033
Deoxynivalenol, 100 µg/mL in acetonitrile	2 mL	34124
Fumonisin B_1, 50 µg/mL in acetonitrile/water	2 mL	34139
Fumonisin B_2, 50 µg/mL in acetonitrile/water	2 mL	34142
Fumonisin B_3, 50 µg/mL in acetonitrile/water	1 mL	32606
Fusarenon X, 100 µg/mL in acetonitrile	2 mL	34130

*Not available in the U.S.A.

Description	Qty.	Cat. No.
Nivalenol, 100 µg/mL in acetonitrile	2 mL	34131
Ochratoxin A, 10 µg/mL in acetonitrile	2 mL	34037
T-2 Toxin, 100 µg/mL in acetonitrile	2 mL	34071*
Zearalenone, 50 µg/mL in acetonitrile	1 mL	46916-U
Zearalenone, 100 µg/mL in acetonitrile	2 mL	34126
Mycotoxin Mixture Standard Solutions for Multi-analyte Detection		
Aflatoxin Mix 4: B_1, 2 µg/mL in acetonitrile G_1, 2 µg/mL in acetonitrile B_2, 0.5 µg/mL in acetonitrile G_2, 0.5 µg/mL in acetonitrile	2 mL 1 mL	34036
Aflatoxin Mix 4: B_1, 20 µg/mL in acetonitrile G_1, 20 µg/mL in acetonitrile B_2, 20 µg/mL in acetonitrile G_2, 20 µg/mL in acetonitrile	2 mL	33415
Japanese Aflatoxin Mix: B_1, 25 µg/mL in acetonitrile G_1, 25 µg/mL in acetonitrile B_2, 25 µg/mL in acetonitrile G_2, 25 µg/mL in acetonitrile	5 x 1 mL	40139-U
Fumonisin Mix: FB_1, 50 µg/mL in acetonitrile/water FB_2, 50 µg/mL in acetonitrile/water	2 mL	34143
B-Trichothecene Mix: DON, 100 µg/mL in acetonitrile NIV, 100 µg/mL in acetonitrile 3-AcDON, 100 µg/mL in acetonitrile 15-AcDON, 100 µg/mL in acetonitrile	2 mL	34134
Certified Matrix Reference Materials for Mycotoxin Analysis (CRMs)		
Compound Feed (Aflatoxin blank) Aflatoxin B_1, <1 µg/kg	50 g	BCR375
Compound Feedingstuff (Aflatoxins, very low level) Aflatoxin B_1, 2.6 µg/kg Aflatoxin B_2, 0.2 µg/kg Aflatoxin G_1, 0.4 µg/kg Aflatoxin G_2, <0.2 µg/kg	2 x 75 g	ERMBE375
Compound Feedingstuff (Aflatoxins, high level) Aflatoxin B_1, 12.9 µg/kg Aflatoxin B_2, 0.68 µg/kg Aflatoxin G_1, 0.5.2 µg/kg	2 x 75 g	ERMBE376
Maize (Zearalenone, very low level) Zearalenone, <5 µg/kg	60 g	ERMBC716
Maize (Zearalenone, low level) Zearalenone, 83 µg/kg	60 g	ERMBC717
Maize Flour (Deoxynivalenol, blank) Deoxynivalenol, <50 µg/kg	150 g	BCR377
Peanut Butter (Aflatoxins, low level) Aflatoxin B_1, <0.2 µg/kg Aflatoxin B_2, <0.2 µg/kg Aflatoxin G_1, <0.2 µg/kg Aflatoxin G_2, <0.2 µg/kg	1 ea.	BCR401R
Wheat (Ochratoxin A, blank) Ochratoxin A, <0.6 µg/kg	55 g	BCR471
Mycotoxin Solutions (CRMs)		
Aflatoxin B_1, 3.79 µg/g in acetonitrile	4 mL	ERMAC057
Aflatoxin B_2, 3.80 µg/g in acetonitrile	4 mL	ERMAC058
Aflatoxin G_1, 3.78 µg/g in acetonitrile	4 mL	ERMAC059
Aflatoxin G_2, 3.80 µg/g in acetonitrile	4 mL	ERMAC060
4-Deoxynivalenol, 25.1 µg/g in acetonitrile	1 ea.	IRMM315
Nivalenol, 24.0 µg/g in acetonitrile	1 ea.	IRMM316
Zearalenone, 9.95 µg/g in acetonitrile	4 mL	ERMAC699

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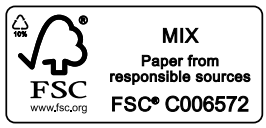

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