

RIDASCREEN® Aflatoxin M1

Art. No. R1121

Product information

- **Reliable**
Extensively tested, excellent performance
- **Sensitive**
Low limit of detection (5 ppt for milk)
- **Easy**
All reagents ready to use
- **Automatable**
Options available



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RIDASCREEN® Aflatoxin M1 is a competitive enzyme immunoassay for the quantitative analysis of aflatoxin M1 in milk and milk powder. Application notes for cheese and butter samples are available on request.

Background

Aflatoxins are carcinogenic and highly toxic metabolites of the fungi species *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin M1 is produced as a metabolite of aflatoxin B1. It is secreted with the milk after feeding of aflatoxin B1 containing feed to lactating cows. As aflatoxin M1 is relatively stable towards the pasteurizing process, not only a comprehensive routine check of the raw materials to be processed is required but also of the final products.

Brief information

Technology:	ELISA
Format:	Microtiter plate with 96 wells (12 strips with 8 wells each)
Sample preparation:	Milk: degrease / Milk powder: reconstitute and degrease
Incubation time:	1 h 15 min
Limit of detection (LOD):	Milk 5 ppt / Milk powder 50 ppt
Detection range:	5 - 80 ppt (ng/kg)
Recovery:	Milk 100 % / Milk powder 116 % (ESM reference material 111 %)
Specificity:	Aflatoxin M1 100% / Aflatoxin M2 < 10 %

Benefits

Sensitive:	Low limit of detection (LOD)
Easy:	Ready-to-use test kit components, simple and efficient sample preparation
Fast:	Only 1 h 15 min test duration
Precise:	Reliable and reproducible results

Test principle

The basis of the test is the antigen-antibody reaction. The wells of the microtiter strips are coated with capture antibodies directed against anti-aflatoxin M1 antibodies. Anti-aflatoxin M1 antibodies are added which are bound by the immobilized capture antibodies. After incubation and washing step, standards or sample solutions are added. After a further incubation with a subsequent washing step, the aflatoxin M1 enzyme conjugate is added. Free and enzyme conjugated aflatoxin M1 compete for the antibody binding sites (competitive enzyme immunoassay). Any unbound enzyme conjugate is then removed in a washing step. Substrate/chromogen is added to the wells and incubated. Bound enzyme conjugate converts the chromogen into a blue product.

The addition of the stop solution leads to a color change from blue to yellow. The measurement is made photometrically at 450 nm and the absorption is inversely proportional to the aflatoxin M1 concentration in the sample.

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Test procedure

Place the required number of wells in the frame.

1		Add 100 µl of antibody solution
15 min	Incubation at room temperature	
2		Wash 3 times with washing buffer Add 100 µl of standard solution or prepared sample
30 min	Incubation at room temperature in the dark	
3		Wash 3 times with washing buffer Add 100 µl of enzyme conjugate
15 min	Incubation at room temperature in the dark	
4		Wash 3 times with washing buffer Add 100 µl substrate/chromogen
15 min	Incubation at room temperature in the dark	
5		Add 100 µl of stop solution Photometrical measurement at 450 nm

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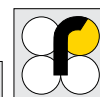
Limit of detection (LOD)

Milk						
Negative samples	Lot 1		Lot 2		Lot 3	
	B/B ₀	c [ppt]	B/B ₀	c [ppt]	B/B ₀	c [ppt]
n = 20	89.8	3.95	95.3	2.69	95.6	2.94
	87.9	4.45	101	<Std 1	94.7	3.17
	96.5	2.47	97.4	2.16	99.9	1.98
	93.6	3.05	98.4	1.93	101.5	<Std 1
	93.1	3.18	94.7	2.86	97.1	2.60
	93.8	3.01	95.3	2.70	98.6	2.24
	93.8	3.02	94.5	2.92	101.0	<Std 1
	92.5	3.30	96.4	2.40	97.5	2.50
	91.2	3.60	97.0	2.26	99.1	2.14
	88.5	4.27	94.6	2.89	99.5	2.05
	96.2	2.53	95.4	2.67	99.8	1.99
	95.0	2.77	94.7	2.86	98.4	2.29
	95.5	2.67	92.9	3.38	96.7	2.67
	94.1	2.94	93.3	3.28	95.3	3.02
	100	<Std 1	91.1	3.97	95.7	2.93
	93.0	3.19	91.7	3.77	95.8	2.91
	94.3	2.92	92.5	3.52	97.5	2.50
	92.2	3.37	91.7	3.75	95.7	2.93
	92.6	3.28	94.6	2.89	97.8	2.43
	91.0	3.65	92.0	3.67	97.6	2.47
Mean		3.24		2.98		2.54
SD		0.54		0.58		0.38
LOD				4.4		

c = concentration

SD = standard deviation

LOD = limit of detection (mean + 3 x SD)



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Recovery

Spiked samples	Milk					
	Lot 1		Lot 2		Lot 3	
	c [ppt]	Recovery [%]	c [ppt]	Recovery [%]	c [ppt]	Recovery [%]
neg	< LOD		< LOD		< LOD	
neg	< LOD		< LOD		< LOD	
neg	< LOD		< LOD		< LOD	
neg	< LOD		< LOD		< LOD	
neg	< LOD		< LOD		< LOD	
neg	< LOD		< LOD		< LOD	
10 ppt	8.86	89	11.17	112	9.78	98
10 ppt	8.38	84	10.96	110	9.62	96
10 ppt	8.35	84	11.07	111	9.96	100
10 ppt	9.18	92	11.04	110	9.65	97
10 ppt	9.98	100	11.32	113	10.55	106
10 ppt	8.76	88	11.57	116	9.91	99
25 ppt	23.5	94	29.18	117	24.64	99
25 ppt	24.05	96	28.75	115	25.08	100
25 ppt	24.32	97	28.33	113	25.24	101
25 ppt	24.27	97	28.25	113	25.17	101
25 ppt	23.21	93	28.67	115	24.71	99
25 ppt	24.59	98	28.67	115	24.83	99
50 ppt	41.57	83	53.96	108	51.01	102
50 ppt	43.38	87	55.47	111	51.21	102
50 ppt	41.63	83	55.47	111	52.46	105
50 ppt	42.12	84	56.18	112	53.54	107
50 ppt	42.05	84	55.13	110	53.65	107
50 ppt	43.54	87	56.18	112	54.88	110
75 ppt	58.09	77	76.36	102	69.96	93
75 ppt	62.12	83	82.55	110	75.78	101
75 ppt	59.85	80	83.91	112	75.78	101
75 ppt	60.03	80	83.57	111	75.96	101
75 ppt	62.73	84	83.91	112	74.36	99
75 ppt	58.78	78	85.65	114	74.53	99
Mean		88		112		101
Mean (total)				100		

c = concentration

LOD = limit of detection

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Limit of detection (LOD)

Milk powder (referring to reconstituted milk)						
Negative samples	Lot 1		Lot 2		Lot 3	
	B/B ₀	c [ppt]	B/B ₀	c [ppt]	B/B ₀	c [ppt]
n = 21	95.6	33.95	97.5	27.29	101.3	<Std 1
	99.7	25.47	94.8	33.55	105.2	<Std 1
	97.6	29.66	96.8	28.89	96.0	27.53
	100.9	<Std 1	96.6	29.39	98.5	22.15
	96.6	31.93	93.8	36.17	92.9	34.96
	98.7	27.37	92.9	38.46	93.5	33.42
	98.9	27.07	92.2	40.37	98.2	22.62
	98.3	28.30	94.9	33.42	97.8	23.49
	92.8	40.67	88.9	49.91	90.3	41.90
	99.4	25.96	95.0	33.02	97.8	23.59
	95.1	35.10	89.7	47.42	93.5	33.30
	95.6	33.95	93.4	37.16	97.6	23.98
	96.7	31.71	94.7	33.96	95.5	28.49
	96.9	31.17	93.3	37.30	92.9	34.84
	95.9	33.39	92.5	39.48	92.3	36.41
	92.0	42.71	90.5	45.16	91.1	39.68
	100.8	<Std 1	95.3	32.49	97.0	25.27
	98.1	28.61	94.6	34.23	95.1	29.46
	96.1	33.05	91.8	41.43	94.9	30.01
	97.3	30.30	93.7	36.45	96.3	26.70
	97.0	30.95	96.7	29.14	93.2	34.12
Mean	31.65		36.41		30.10	
SD	4.52		6.05		5.97	
LOD	49					

c = concentration

SD = standard deviation

LOD = limit of detection (mean + 3 x SD)

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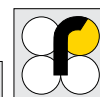
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Recovery

Milk powder (referring to reconstituted milk)						
Spiked samples	Lot 1		Lot 2		Lot 3	
	c [ppt]	Recovery [%]	c [ppt]	Recovery [%]	c [ppt]	Recovery [%]
neg	< LOD		< LOD		< LOD	
neg	< LOD		< LOD		< LOD	
neg	< LOD		< LOD		< LOD	
neg	< LOD		< LOD		< LOD	
100 ppt	11.00	110	11.56	116	11.51	115
100 ppt	11.45	114	11.46	115	11.57	116
100 ppt	11.63	116	11.76	118	11.71	117
100 ppt	11.39	114	11.49	115	11.43	114
100 ppt	11.24	112	11.24	112	10.79	108
100 ppt	11.16	112	11.49	115	10.66	107
250 ppt	27.43	110	30.95	124	27.28	109
250 ppt	28.05	112	29.97	120	25.53	102
250 ppt	28.72	115	31.69	127	27.65	111
250 ppt	27.51	110	30.73	123	25.99	104
250 ppt	27.66	111	30.03	120	25.65	103
250 ppt	26.76	107	29.45	118	25.32	101
500 ppt	58.90	118	67.30	135	59.04	118
500 ppt	59.83	120	64.91	130	58.38	117
500 ppt	61.03	122	69.30	139	60.73	121
500 ppt	58.02	116	66.81	134	58.87	118
500 ppt	58.46	117	70.08	140	59.37	119
500 ppt	60.06	120	66.33	133	61.08	122
750 ppt	84.88	113	89.47	119	78.69	105
750 ppt	76.38	102	88.44	118	74.99	100
750 ppt	90.29	120	97.05	129	82.58	110
750 ppt	82.09	109	95.93	128	78.22	104
750 ppt	87.34	116	96.30	128	80.13	107
750 ppt	83.28	111	92.29	123	74.10	99
Mean		114		124		110
Mean (total)				116		

c = concentration

LOD = limit of detection



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Recovery

Milk powder (referring to reconstituted milk)							
ERM® reference material	Target value [ppt]	Lot 1		Lot 2		Lot 3	
		c [ppt]	Recovery [%]	c [ppt]	Recovery [%]	c [ppt]	Recovery [%]
ERM 282	neg	< LOD		< LOD		< LOD	
ERM 282	neg	< LOD		< LOD		< LOD	
ERM 282	neg	< LOD		< LOD		< LOD	
ERM 282	neg	< LOD		< LOD		< LOD	
ERM 282	neg	< LOD		< LOD		< LOD	
ERM 282	neg	< LOD		< LOD		< LOD	
ERM 283	111	124	112	133	120	128	115
ERM 283	111	121	109	133	120	125	113
ERM 283	111	124	111	135	122	132	119
ERM 283	111	120	109	127	114	127	115
ERM 283	111	120	108	126	114	123	111
ERM 283	111	122	110	130	117	122	110
ERM 284	444	461	104	497	112	494	111
ERM 284	444	458	103	509	115	500	113
ERM 284	444	464	104	495	112	493	111
ERM 284	444	450	101	483	109	470	106
ERM 284	444	442	100	485	109	472	106
ERM 284	444	465	105	492	111	483	109
Mean			106		114		111
Mean (total)					111		

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Repeatability and reproducibility

Milk powder (referring to reconstituted milk)				
Participant	1	2	3	4
Lot	1	2	3	4
Spiked sample [250ppt]	c [ppt]	c [ppt]	c [ppt]	c [ppt]
Sample 1	229.7	212.1	206.0	234.6
Sample 2	221.9	262.8	232.1	223.5
Sample 3	214.6	260.9	217.0	249.4
Sample 4	212.6	245.3	204.4	–
Sample 5	210.6	247.4	225.0	–
Sample 6	208.9	240.4	222.7	–
Sample 7	215.8	289.4	216.6	–
Sample 8	–	–	256.8	–
Standard deviation of repeatability (sr)	16.9			
Repeatability (r)	47.3			
Standard deviation of reproducibility (sR)	22.7			
Reproducibility (R)	66.1			

c = concentration

Remark

RIDASCREEN® Aflatoxin M1 corresponds with specifications of ISO Norm 14675:2003(E).

Data calculated from in-house QC testing over several lots and months with different technicians using one stable sample. Collaborative data not available until the time point of finalization of this document.

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Related products

Product	Description	No. of Tests / Amount	Code No.
Aflatoxin			
ELISA Microtiter Plates			
RIDASCREEN®FAST Aflatoxin M1	Enzyme immunoassay for quantitative analysis of aflatoxin M1 in milk and milk powder Detection limit: < 125 ppt	48 determinations Incubation time: 15 min	R5812
Immunoaffinity Columns			
AFLAPREP® M	Immunoaffinity columns for sample clean-up prior to the analysis of aflatoxin M1 using HPLC	25 columns (1 ml format)	RBRP04
AFLAPREP® M WIDE	Immunoaffinity columns for sample clean-up prior to the analysis of aflatoxin M1 using HPLC	10 columns (3 ml format) 50 columns (3 ml format)	RBRP124 RBRP124B
Standard			
liquid			
AFLATOXINM1 Standard	Aflatoxin M1 standard solution at a concentration of 1000 ng/ml in acetonitrile	6 ml	RBRP42
Trilogy® Liquid Standard Aflatoxin M1	Aflatoxin M1; 0.5 µg/ml acetonitrile	2 ml	TSL-143
Standard			
dried			
Trilogy® Dried Standard Aflatoxin M1	Aflatoxin M1	1 µg/ml after reconstitution	TS-130

For further information or orders please contact R-Biopharm AG:

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