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Validation Report

Beta-lactoglobulin ELISA Kit II (Cat.# M2112)

Sandwich enzyme immunoassay for the quantitative determination of milk proteins in processed and unprocessed foods

Limit of Detection: 0.31 μ g milk protein/g food

Standard Range: 0.31-20 μ g milk protein/g food

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1. Scope

The **Beta-lactoglobulin ELISA Kit II** is sandwich enzyme immunoassay for the quantitative determination of milk proteins in processed and unprocessed foods.

2. Precision

2.1. Intra-Assay Variation

The intra-assay variation was determined by testing three controls in 3-fold replicates.

Extraction : Overnight Extraction Method

Replicate	control 1	control 2	control 3
1	4.57	8.04	8.19
2	4.57	7.86	8.15
3	4.69	8.17	8.31
Mean	4.61	8.02	8.22
SD	0.07	0.16	0.08
CV(%)	1.5%	1.9%	1.0%

2.2. Inter-Assay Variation

The inter-assay variation was determined by testing three controls in three different test runs of the same lot of kit.

Extraction : Overnight Extraction Method

Assay No.	control 1	control 2	control 3
1	5.41	7.63	7.66
2	5.55	7.89	7.86
3	5.38	8.02	8.22
Mean	5.42	7.70	7.80
SD	0.09	0.20	0.28
CV(%)	2.7%	3.5%	4.8%

3. Recovery

3.1 Incurred foods

For recovery experiments, milk incurred foods were prepared with 10ppm protein of milk contamination.

Extraction : Overnight Extraction Method

Food samples	Heating condition	Actual Concentration [ppm]	Recovery [%]
Orange Juice	Heated at 90°C for 10min	7.6	76%
Jelly	Heated up to reach 90°C	7.7	77%
tomato sauce	Heated at 85°C for 30min	7.9	79%

Extraction : Short Time Extraction Method

Food samples	Heating condition	Actual Concentration [ppm]	Recovery [%]
Orange Juice	Heated at 90°C for 10min	7.7	77%
Jelly	Heated up to reach 90°C	7.5	75%
tomato sauce	Heated at 85°C for 30min	7.9	79%

3.2 Spiked foods

For recovery experiments, different sample matrices were spiked with -
 Reference Material for the Detection of Milk Protein (Based on Skim Milk Powder, Product code: SMP,
 Art. No. : MQA 092014) .

The contamination levels were 0.5, 1, 2.5, 5 ppm.

Each extraction option of the BLG ELISA Kit II was tested by 3 individual extracted samples per level.

Extraction : Overnight Extraction Method & Short Time Extraction Method

[Water]: Overnight Extraction

Target Value [ppm]	Actual Conc. [ppm]	Recovery [%]
5.0	3.9	78%
2.5	1.8	73%
1.0	0.8	76%
0.5	0.4	71%
	Mean	75%

[Water]: Short time Extraction

Target Value [ppm]	Actual Conc. [ppm]	Recovery [%]
5.0	4.4	87%
2.5	2.0	78%
1.0	0.7	71%
0.5	0.3	63%
	Mean	75%

[Wine]: Overnight Extraction

Target Value [ppm]	Actual Conc. [ppm]	Recovery [%]
5.0	4.1	82%
2.5	1.7	69%
1.0	0.7	74%
0.5	0.3	68%
	Mean	73%

[Wine]: Short time Extraction

Target Value [ppm]	Actual Conc. [ppm]	Recovery [%]
5.0	4.1	83%
2.5	1.8	72%
1.0	0.8	75%
0.5	0.3	69%
	Mean	75%

[Lemonade]: Overnight Extraction

Target Value [ppm]	Actual Conc. [ppm]	Recovery [%]
5.0	4.0	80%
2.5	1.6	66%
1.0	0.7	75%
0.5	0.3	65%
	Mean	72%

[Lemonade]: Short time Extraction

Target Value [ppm]	Actual Conc. [ppm]	Recovery [%]
5.0	4.1	81%
2.5	1.7	69%
1.0	0.7	73%
0.5	0.3	65%
	Mean	72%

[Juice]: Overnight Extraction

Target Value [ppm]	Actual Conc. [ppm]	Recovery [%]
5.0	3.9	78%
2.5	1.7	69%
1.0	0.8	75%
0.5	0.3	66%
	Mean	72%

[Juice]: Short time Extraction

Target Value [ppm]	Actual Conc. [ppm]	Recovery [%]
5.0	4.1	82%
2.5	1.8	72%
1.0	0.7	71%
0.5	0.3	66%
	Mean	73%

[Chocolate]: Overnight Extraction

Target Value [ppm]	Actual Conc. [ppm]	Recovery [%]
5.0	4.8	96%
2.5	2.2	89%
1.0	0.7	68%
0.5	0.4	84%
	Mean	84%

[Chocolate]: Short time Extraction

Target Value [ppm]	Actual Conc. [ppm]	Recovery [%]
5.0	4.3	87%
2.5	2.0	82%
1.0	0.8	83%
0.5	0.4	87%
	Mean	84%

[Biscuit]: Overnight Extraction

Target Value [ppm]	Actual Conc. [ppm]	Recovery [%]
5.0	4.4	88%
2.5	1.9	75%
1.0	0.8	77%
0.5	0.3	70%
	Mean	78%

[Biscuit]: Short time Extraction

Target Value [ppm]	Actual Conc. [ppm]	Recovery [%]
5.0	4.0	81%
2.5	1.9	76%
1.0	0.9	90%
0.5	0.4	73%
	Mean	80%

4. Analytical Sensitivity

4.1 Sample diluent

For determination of the analytical sensitivity, sample diluent was assayed in 8-fold replicates.

After identification of possible outliers the OD mean and standard deviation was calculated.

The corresponding concentration of the OD mean + 3 x standard deviation was defined as limit of detection and OD mean + 10 x standard deviation was defined as limit of quantification.

Standard curve: mAbs (450nm / 620nm)

STD (ppb)	Ave.	CV%
0	0.020	4.5
0.78	0.049	2.6
1.56	0.080	2.1
3.13	0.142	3.2
6.25	0.262	3.0
12.5	0.502	4.6
25	0.986	3.5
50	1.813	3.9

Blank	0 ppm A
1	0.021
2	0.019
3	0.021
4	0.020
5	0.019
6	0.019
7	0.020
8	0.019
Ave.	0.020
SD	0.001

Reversed quantitative value:
Re-calculate the blank mAbs
using the linear std curve.

*Calculated by linear regression

LOD	0.032 ppm	Reversed quant value +3SD
LOQ	0.095 ppm	Reversed quatn value +10SD

4.2 Matrices

For determination of the analytical sensitivity, different sample matrices were assayed in 10-fold replicates.

After identification of possible outliers the OD mean and standard deviation was calculated.

The corresponding concentration of the OD mean + 3 x standard deviation was defined as limit of detection and OD mean + 10 x standard deviation was defined as limit of quantification.

Extraction: Overnight Extraction

Primary Food Matrices	Water	Wine	Lemonade	Juice	Chocolate	Biscuit
1	0.02	0.02	0.02	0.02	0.04	0.02
2	0.02	0.02	0.02	0.02	0.05	0.02
3	0.02	0.02	0.02	0.02	0.05	0.02
4	0.02	0.02	0.02	0.02	0.04	0.02
5	0.02	0.02	0.02	0.02	0.05	0.02
6	0.02	0.02	0.02	0.02	0.05	0.02
7	0.02	0.02	0.02	0.02	0.05	0.02
8	0.02	0.02	0.02	0.02	0.05	0.02
9	0.02	0.02	0.02	0.02	0.05	0.02
10	0.02	0.02	0.02	0.02	0.05	0.02
Ave	0.02	0.02	0.02	0.02	0.05	0.02
SD	0.00	0.00	0.00	0.00	0.00	0.00
Ave+3SD	0.02	0.02	0.02	0.02	0.06	0.02
Ave+10SD	0.03	0.04	0.03	0.03	0.07	0.03

Extraction: Short Time Extraction

Primary Food Matrices	Water	Wine	Lemonade	Juice	Chocolate	Biscuit
1	0.02	0.02	0.02	0.02	0.04	0.03
2	0.02	0.02	0.03	0.02	0.04	0.03
3	0.02	0.02	0.02	0.02	0.04	0.02
4	0.02	0.02	0.02	0.02	0.04	0.02
5	0.02	0.02	0.02	0.02	0.04	0.02
6	0.02	0.02	0.02	0.02	0.04	0.02
7	0.02	0.02	0.02	0.02	0.04	0.02
8	0.02	0.02	0.02	0.02	0.04	0.02
9	0.02	0.02	0.02	0.02	0.04	0.02
10	0.02	0.02	0.03	0.02	0.05	0.02
Ave	0.02	0.02	0.02	0.02	0.04	0.02
SD	0.00	0.00	0.00	0.00	0.00	0.00
Ave+3SD	0.03	0.03	0.03	0.03	0.05	0.03
Ave+10SD	0.04	0.04	0.05	0.04	0.07	0.05

5. Cross-Reactivity

For the following foods, no cross-reactivity (results<LOQ) could be detected.

Unit : μg milk protein/g food

Egg	<0.31
Milk	>20
Skim milk	>20
Wheat	<0.31
Barley	<0.31
Rye	<0.31
Oats	<0.31
Soy bean	<0.31
Corn flour	<0.31
Peanut	<0.31
Almond (Roasted)	<0.31
Cashew (Roasted)	<0.31
Macadamia (Roasted)	<0.31
Pistachio (Roasted)	<0.31
Walnut (Roasted)	<0.31
Sesame (Roasted)	<0.31
Black pepper	<0.31
Red pepper	<0.31
Cumin	<0.31
Coriander	<0.31
Poppy seed	<0.31
Shrimp	<0.31
Crab	<0.31
Squid	<0.31
Beef	<0.31
Pork	<0.31
Chicken	<0.31

6. Criteria for the standard curve

	Criteria
1) the blank absorbance value	≤ 0.1
2) the absorbance value of 50ng/mL※1	≥ 1.0
3) R ² value※2	≥ 0.99
4) B/B0 (= 50ng/mL absorbance value / blank absorbance value)	≥ 10

※1 The incubation temperature of ELISA is all 25°C.

※2 R² value by using 4-parameter analysis on ELISA data.

4-Parameter fit: $Y=(A-D)/(1+(X/C)^B)+D$