Date of issue: August 13th

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 µg milk protein/g food

Standard Range: 0.31-20 µg 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	10.11	3.80	2.23
2	10.44	3.87	2.27
3	11.01	3.99	2.32
Mean	10.52	3.89	2.27
SD	0.46	0.10	0.05
CV(%)	4.3%	2.5%	2.0%

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	10.53	3.89	2.20
2	10.10	3.85	2.29
3	10.52	3.88	2.27
Mean	10.38	3.87	2.25
SD	0.25	0.02	0.05
CV(%)	2.4%	0.5%	2.1%

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℃ for 10min	8.3	83%
Jelly	Heated up to reach 90℃	5.2	52%
Strawberry jam	Boiled until it has	7.2	72%

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3.2 Spiked foods

For recovery experiments, different sample matrices were spiked with -
Refarence 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	4.7	93%
2.5	2.1	85%
1.0	0.7	74%
0.5	0.3	61%
	Mean	78%

[Water]: Short time Extraction

Target Value [ppm]	Actual Conc. [ppm]	Recovery [%]
5.0	4.3	87%
2.5	2.1	82%
1.0	0.6	62%
0.5	0.3	50%
	Mean	70%

[Wine]: Overnight Extraction

Target Value [ppm]	Actual Conc. [ppm]	Recovery [%]
5.0	3.8	76%
2.5	1.8	73%
1.0	0.5	51%
0.5	0.2	42%
	Mean	60%

[Wine]: Short time Extraction

Target Value [ppm]	Actual Conc. [ppm]	Recovery [%]
5.0	4.0	80%
2.5	2.0	82%
1.0	0.7	67%
0.5	0.2	47%
	Mean	69%

[Lemonade]: Overnight Extraction

Target Value [ppm]	Actual Conc. [ppm]	Recovery [%]
5.0	3.8	77%
2.5	1.8	72%
1.0	0.5	52%
0.5	0.2	35%
	Mean	59%

[Lemonade]: Short time Extraction

Target Value [ppm]	Actual Conc. [ppm]	Recovery [%]
5.0	4.3	85%
2.5	1.9	77%
1.0	0.6	61%
0.5	0.3	60%
	Mean	71%

[Juice]: Overnight Extraction

Target Value [ppm]	Actual Conc. [ppm]	Recovery [%]
5.0	3.8	75%
2.5	1.7	66%
1.0	0.5	49%
0.5	0.2	36%
	Mean	57%

[Juice]: Short time Extraction

Target Value [ppm]	Actual Conc. [ppm]	Recovery [%]
5.0	4.0	80%
2.5	1.8	73%
1.0	0.6	61%
0.5	0.2	44%
	Mean	64%

[Chocolate]: Overnight Extraction

Target Value [ppm]	Actual Conc. [ppm]	Recovery [%]
5.0	4.3	86%
2.5	2.1	82%
1.0	0.7	69%
0.5	0.3	60%
	Mean	74%

[Chocolate]: Short time Extraction

Target Value [ppm]	Actual Conc. [ppm]	Recovery [%]
5.0	4.1	82%
2.5	2.2	88%
1.0	0.7	67%
0.5	0.3	57%
	Mean	73%

[Biscuit]: Overnight Extraction

Target Value [ppm]	Actual Conc. [ppm]	Recovery [%]
5.0	3.7	73%
2.5	1.7	66%
1.0	0.5	50%
0.5	0.1	29%
	Mean	54%

[Biscuit]: Short time Extraction

Target Value [ppm]	Actual Conc. [ppm]	Recovery [%]
5.0	4.0	80%
2.5	1.9	78%
1.0	0.8	78%
0.5	0.3	69%
	Mean	76%

4. Analytical Sensitivity

4.1 Sample diluent

For determination of the analytical sensitivity, sample diluent was assayed in 4-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.

Replicate	0ng/mL(OD)
1	0.012
2	0.008
3	0.010
4	0.011
Mean	0.010
SD	0.002
Limit of Detection	0.31µg milk protein/g food
Limit of Quantification	0.31µg milk protein/g food

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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 devation was defined as limit of detection and OD mean + 10 x standard devation was defined as limit of quantification.

Extraction: Overnight Extraction

Primary Food Matrices	Water	Wine	Lemonade	Juice	Chocolate	Buiscuit
1	0.00	0.03	0.00	0.03	0.01	0.02
2	0.00	0.00	0.04	0.01	0.02	0.00
3	0.00	0.00	0.01	0.00	0.02	0.00
4	0.01	0.04	0.04	0.03	0.02	0.02
5	0.01	0.00	0.04	0.00	0.02	0.00
6	0.00	0.01	0.02	0.11	0.02	0.00
7	0.00	0.00	0.00	0.01	0.04	0.00
8	0.00	0.02	0.00	0.03	0.01	0.00
9	0.00	0.00	0.01	0.03	0.04	0.00
10	0.02	0.00	0.05	0.02	0.02	0.00
Ave	0.01	0.01	0.02	0.03	0.02	0.00
SD	0.01	0.01	0.02	0.03	0.01	0.01
Ave+3SD	0.03	0.05	0.08	0.12	0.06	0.03
Ave+10SD	0.08	0.15	0.22	0.33	0.14	0.09

Extraction: Short Time Extraction

Primary Food Matrices	Water	Wine	Lemonade	Juice	Chocolate	Buiscuit
1	0.00	0.00	0.02	0.00	0.02	0.00
2	0.04	0.00	0.06	0.01	0.02	0.00
3	0.02	0.00	0.05	0.02	0.01	0.00
4	0.00	0.00	0.03	0.02	0.01	0.00
5	0.00	0.00	0.00	0.05	0.02	0.00
6	0.02	0.00	0.02	0.04	0.02	0.02
7	0.00	0.00	0.02	0.07	0.01	0.01
8	0.01	0.00	0.05	0.06	0.01	0.00
9	0.01	0.00	0.02	0.01	0.01	0.00
10	0.04	0.00	0.09	0.02	0.01	0.00
Ave	0.01	0.00	0.04	0.03	0.01	0.00
SD	0.01	0.00	0.02	0.02	0.01	0.01
Ave+3SD	0.06	0.01	0.11	0.10	0.03	0.03
Ave+10SD	0.16	0.02	0.29	0.25	0.08	0.08

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

* The latest food reactivity data is listed on our web page: http://www.miobs-e.com/index.html

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℃.

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

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

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