Validation Report

Beta-lactoglobulin ELISA Kit Ⅱ

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

Limit of Detection: 0.31μg 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

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 hasevaporated	7.2	72%

4. Analytical Sensitivity

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

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℃.
※2 R^2 value by using 4-parameter analysis on ELISA data.
4-Parameter fit: Y=(A-D)/(1+(X/C)^B)+D