Validation Report

Egg (ovalbumin) ELISA Kit Ⅱ

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

Limit of Detection: 0.31μg egg protein/g food

Standard Range: 0.31-20 μg egg protein/g food

Morinaga Institute of Biological Science, Inc.

Sachiura 2-1-16, Kanazawa-Ku,
Yokohama-Shi,
236-0003, Japan

TEL: +81-45-791-7674
FAX: +81-45-791-7675

E-mail for inquiries: info@miobs.com

1. Scope

The **Egg (ovalbumin) ELISA Kit Ⅱ** is sandwich enzyme immunoassay for the quantitative determination of egg proteins in processed and unprocessed foods.

2. Precision

2.1. Intra-Assay Variation

The intra-assay variation was determined by testing three controls in 5-fold replicates.
Extraction : Overnight Extraction Method

Replicate	control 1	control 2	control 3
1	7.85	8.20	10.12
2	8.04	7.01	10.12
3	8.64	8.38	10.62
4	8.60	8.11	10.72
5	7.97	7.13	9.98
Mean	8.22	7.77	10.31
SD	0.37	0.64	0.33
CV(%)	4.5%	8.3%	3.2%

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	6.91	7.67	10.00
2	7.85	8.20	10.12
3	8.41	8.55	10.52
Mean	7.72	8.14	10.21
SD	0.76	0.44	0.27
CV(%)	9.8%	5.4%	2.7%

3. Recovery

For recovery experiments, egg incurred foods were prepared with 10ppm protein of egg contamination.
Extraction : Overnight Extraction Method

Food samples	Heating condition	Actual Concentration (ppm)	Recovery (%)
Orange Juice	Heated at 90℃ for 10min	8.7	87%
Jelly	Heated up to reach 90℃	9.3	93%
Cake	Heated for 2min in a microwave oven	6.8	68%
Porridge	Cooked by rice cooker	7.4	74%

For another experiment, two sample matrices were spiked with egg to obtain various final concentrations before extraction.
Extraction : Overnight Extraction Method

Jelly

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
10	9.3	93%
5	4.5	90%
1	0.7	70%

Cooked rice

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
10	9.2	92%
5	4.6	92%
1	0.8	80%

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	0.025
2	0.030
3	0.030
4	0.029
Mean	0.029
SD	0.002
Limit of Detection	0.31µg egg protein/g food
Limit of Quantification	0.31µg egg protein/g food

5. Cross-Reactivity

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

Unit: µg egg protein/g food

Egg	＞20
Milk	＜0.31
Skim milk	＜0.31
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