Date of issue: March 19th

Validation Report

Sesame ELISA Kit Ⅱ (Cat.# M2121)

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

Limit of Detection: 0.16 μg sesame protein/g food

Standard Range: 0.16-10 μg sesame 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_e@morinaga.com

1. Scope

The **Sesame ELISA Kit Ⅱ** is a sandwich enzyme immunoassay for the quantitative determination of sesame 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	control 4
1	7.98	9.61	6.14	8.19
2	7.99	9.65	6.02	7.77
3	8.59	10.54	6.45	8.16
Mean	8.18	9.93	6.20	8.04
SD	0.35	0.53	0.22	0.23
CV(%)	4.3%	5.3%	3.5%	2.9%

2.2. Inter-Assay Variation

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

Assay No.	control 1	control 2	control 3	control 4
1	7.99	9.65	6.02	7.77
2	7.48	9.49	5.64	7.83
3	7.99	9.71	5.86	7.68
Mean	7.82	9.62	5.84	7.76
SD	0.29	0.11	0.19	0.08
CV(%)	3.7%	1.2%	3.3%	1.0%

3. Recovery

3.1 Incurred foods

In preparation

Morinaga Institute of Biological Science, Inc.
SACHIURA 2-1-16, KANAZAWA-KU, YOKOHAMA-SHI 236-0003, JAPAN TEL:+81-45-791-7673 / FAX:+81-45-791-7675
http://www.miobs-e.com/index.html

3.2 Spiked foods

For recovery experiments different sample matrices were spiked with sesame powder.
The contamination levels were 0.25, 1, 2.5, 5 ppm.
Each extraction option of the Sesame ELISA Kit Ⅱ was tested by 3 individual extracted samples per contamination level, and a mean value was taken.
Extraction : Overnight Extraction Method & Short Time Extraction Method

Overnight Extraction Method

Water

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.00	4.95	99%
2.50	2.43	97%
1.00	0.92	92%
0.25	0.23	93%
	Mean	95%

White pepper

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.00	4.5	89%
2.50	2.1	82%
1.00	0.8	80%
0.25	0.2	76%
	Mean	82%

Dressing

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.00	4.28	86%
2.50	1.98	79%
1.00	0.74	74%
0.25	0.17	68%
	Mean	77%

Cookie

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.00	4.39	88%
2.50	2.40	96%
1.00	0.72	72%
0.25	0.18	74%
	Mean	82%

Short Time Extraction Method

Water

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.00	5.07	101%
2.50	2.72	109%
1.00	1.13	113%
0.25	0.24	97%
	Mean	105%

White pepper

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.00	4.7	94%
2.50	2.5	101%
1.00	1.0	95%
0.25	0.2	99%
	Mean	97%

Dressing

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.00	4.70	94%
2.50	2.47	99%
1.00	0.91	91%
0.25	0.23	91%
	Mean	94%

Cookie

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.00	5.22	104%
2.50	2.54	101%
1.00	0.93	93%
0.25	0.20	81%
	Mean	95%

Ice Cream

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.00	4.16	83%
2.50	2.34	94%
1.00	0.83	83%
0.25	0.23	92%
	Mean	88%

Ice Cream

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.00	4.40	88%
2.50	2.46	98%
1.00	0.89	89%
0.25	0.21	85%
	Mean	90%

Pasta

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.00	4.27	85%
2.50	1.96	78%
1.00	0.83	83%
0.25	0.20	81%
	Mean	82%

Pasta

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.00	5.07	101%
2.50	2.70	108%
1.00	1.00	100%
0.25	0.24	95%
	Mean	101%

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.

In preparation

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 Method

Primary Food Matrices	Water	White pepper	Dressing	Cookie	Ice Cream	Pasta
1	0.03	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	0.02
3	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00	0.01
6	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	0.01	0.00
8	0.01	0.00	0.00	0.01	0.00	0.00
9	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.01	0.00	0.00
Mean, ppm	0.00	0.00	0.00	0.00	0.00	0.00
SD	0.01	0.00	0.00	0.00	0.00	0.00
Limit of Detection, ppm	0.03	<0.01	<0.01	0.01	0.01	0.02
Limit of Quantification, ppm	0.09	<0.01	<0.01	0.03	0.04	0.05

Extraction : Short time Extraction Method

Primary Food Matrices	Water	White pepper	Dressing	Cookie	Ice Cream	Pasta
1	0.00	0.00	0.00	0.00	0.00	0.01
2	0.00	0.00	0.00	0.00	0.00	0.01
3	0.00	0.00	0.01	0.00	0.00	0.01
4	0.00	0.01	0.01	0.00	0.01	0.01
5	0.00	0.00	0.00	0.00	0.00	0.01
6	0.00	0.00	0.00	0.00	0.00	0.01
7	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.01
9	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.01	0.00	0.01
Mean	0.00	0.00	0.00	0.00	0.00	0.01
SD	0.00	0.00	0.00	0.00	0.00	0.00
Limit of Detection, ppm	<0.01	0.01	0.01	0.01	0.01	0.02
Limit of Quantification, ppm	<0.01	0.03	0.02	0.02	0.04	0.04

5. Cross-Reactivity

For the following foods, no cross-reactivity (results<LOQ) were detected.
Unit: μg sesame protein/g food

Extraction: Overnight method

Rice powder	< 0.16	Hazel (roasted)	< 0.16	Peacan nut (raw)	< 0.16	Basil	< 0.16
Buckwheat	< 0.16	Almond (roasted)	< 0.16	Brazil nut	< 0.16	Red pepper	< 0.16
Wheat	< 0.16	Macadamia (roasted)	< 0.16	sunflower seed (raw)	< 0.16		
Soy	< 0.16	Cashew (roasted)	< 0.16	Pine nut	< 0.16		
Peanut	< 0.16	Pistachio (roasted)	< 0.16	lentil	< 0.16		
Shrimp	< 0.16	Walnut (roasted)	< 0.16	Green pea	< 0.16		
Crub	< 0.16	Peacan nut (roasted)	< 0.16	Lupin beans	< 0.16		
Egg	< 0.16	Hazel (raw)	< 0.16	chickpea	< 0.16		
Egg (boiled)	< 0.16	Almond (raw)	< 0.16	Cumin	< 0.16		
Dried Egg	< 0.16	Macadamia (raw)	< 0.16	Coriander	< 0.16		
Milk	< 0.16	Cashew (raw)	< 0.16	Black pepper	< 0.16		
Skim milk	< 0.16	Pistachio (raw)	< 0.16	White pepper	< 0.16		
Corn flour	< 0.16	Walnut (raw)	< 0.16	Turmeric	< 0.16		

Extraction : Short time Extraction Method

Rice powder	< 0.16	Hazel (roasted)	< 0.16	Peacan nut (raw)	< 0.16	Basil	< 0.16
Buckwheat	< 0.16	Almond (roasted)	< 0.16	Brazil nut	< 0.16	Red pepper	< 0.16
Wheat	< 0.16	Macadamia (roasted)	< 0.16	sunflower seed (raw)	< 0.16		
Soy	< 0.16	Cashew (roasted)	< 0.16	Pine nut	< 0.16		
Peanut	< 0.16	Pistachio (roasted)	< 0.16	lentil	< 0.16		
Shrimp	< 0.16	Walnut (roasted)	< 0.16	Green pea	< 0.16		
Crub	< 0.16	Peacan nut (roasted)	< 0.16	Lupin beans	< 0.16		
Egg	< 0.16	Hazel (raw)	< 0.16	chickpea	< 0.16		
Egg (boiled)	< 0.16	Almond (raw)	< 0.16	Cumin	< 0.16		
Dried Egg	< 0.16	Macadamia (raw)	< 0.16	Coriander	< 0.16		
Milk	< 0.16	Cashew (raw)	< 0.16	Black pepper	< 0.16		
Skim milk	< 0.16	Pistachio (raw)	< 0.16	White pepper	< 0.16		
Corn flour	< 0.16	Walnut (raw)	< 0.16	Turmeric	< 0.16		

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

Morinaga Institute of Biological Science, Inc.
SACHIURA 2-1-16, KANAZAWA-KU, YOKOHAMA-SHI 236-0003, JAPAN TEL:+81-45-791-7673 / FAX:+81-45-791-7675
http://www.miobs-e.com/index.html