

Validation Report

High Sensitive Peanut ELISA Kit II (Cat.# M2120)

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

Limit of Detection : 0.2 µg NIST peanut butter SRM2387 protein/g food

Standard Range : 0.2-12.8 µg NIST peanut butter SRM2387 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.co.jp

1. Scope

The **High Sensitive Peanut ELISA Kit II** is sandwich enzyme immunoassay for the quantitative determination of peanut proteins in processed and unprocessed foods.

2. Precision

2.1. Intra-Assay Variation

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

Extraction : Overnight Extraction Method

Assay No.	control 1	control 2	control 3	control 4
1	4.84	6.59	8.24	3.99
2	4.76	6.60	8.22	4.03
3	4.91	6.75	8.25	3.96
Mean	4.84	6.65	8.24	3.99
SD	0.07	0.09	0.01	0.03
CV%	1.5%	1.4%	0.2%	0.9%

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
day 1	4.95	6.83	8.26
day 2	5.00	6.97	8.64
day 3	4.65	6.63	8.28
Mean	4.87	6.81	8.39
SD	0.19	0.17	0.21
CV%	3.8%	2.5%	2.5%

3. Recovery

3.1 Incurred foods

For recovery experiments, peanut incurred foods were prepared with 6.4ppm NIST peanut protein.

Extraction : Overnight Extraction Method

Food samples	Heating condition	Actual Concentration [ppm]	Recovery [%]
Juice	Heated at 90°C for 10min	4.8	74%
Jelly	Heated up to reach 90°C	5.8	91%
Strawberry jam	Heated at 94°C for 4min	8.1	126%
Tomato stew	Heated at 123°C for 12min 3sec	3.3	52%
Retort food 1	Heated at 123°C for 12min	3.7	57%
Retort food 2	Heated at 123°C for 12min 25sec	3.5	54%

Extraction : Short Time Extraction Method

Food samples	Heating condition	Actual Concentration [ppm]	Recovery [%]
Juice	Heated at 90°C for 10min	4.4	69%
Jelly	Heated up to reach 90°C	5.9	92%
Strawberry jam	Heated at 94°C for 4min	7.8	122%
Tomato stew	Heated at 123°C for 12min 3sec	3.2	51%
Retort food 1	Heated at 123°C for 12min	4.0	63%
Retort food 2	Heated at 123°C for 12min 25sec	3.8	59%

3.2 Spiked foods

For recovery experiments different sample matrices were spiked with NIST peanut butter SRM2387. The contamination level was 6.4 and 0.3, 1, 2.5 and 5 ppm.

Extraction : Overnight Extraction Method & Short Time Extraction Method

Extraction : Overnight Extraction Method

Food matrices	Actual Concentration [ppm]	Recovery [%]
Drecing	6.4	99%
Sauce	6.2	96%
Ice cream	5.7	88%
Pasta	5.5	86%
Cookie	5.4	84%
Water	5.4	85%

Extraction : Short Time Extraction Method

Food matrices	Actual Concentration [ppm]	Recovery [%]
Drecing	6.4	100%
Sauce	6.4	99%
Ice cream	5.7	89%
Pasta	5.6	87%
Cookie	6.3	98%
Water	5.9	93%

[Overnight Extraction Method]

Water

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	5.8	117%
2.5	2.9	116%
1.0	1.2	117%
0.3	0.3	110%
	Mean	115%

[Short Time Extraction Method]

Water

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	6.3	126%
2.5	3.0	122%
1.0	1.2	119%
0.3	0.4	117%
	Mean	121%

Chocolate

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	5.1	102%
2.5	2.5	101%
1.0	1.0	104%
0.3	0.3	99%
	Mean	101%

Chocolate

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	4.6	91%
2.5	2.2	89%
1.0	0.9	92%
0.3	0.3	94%
	Mean	92%

Ice Creame

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	5.3	106%
2.5	2.5	101%
1.0	1.0	103%
0.3	0.3	90%
	Mean	100%

Ice Creame

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	6.1	122%
2.5	3.1	126%
1.0	1.2	125%
0.3	0.3	114%
	Mean	122%

Sauce

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	5.9	119%
2.5	2.8	113%
1.0	1.1	112%
0.3	0.3	108%
	Mean	113%

Sauce

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	6.6	131%
2.5	3.3	131%
1.0	1.3	133%
0.3	0.4	126%
	Mean	130%

Dressing

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	5.3	107%
2.5	2.6	104%
1.0	1.0	100%
0.3	0.3	95%
	Mean	102%

Dressing

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	6.1	121%
2.5	2.9	116%
1.0	1.2	124%
0.3	0.4	127%
	Mean	122%

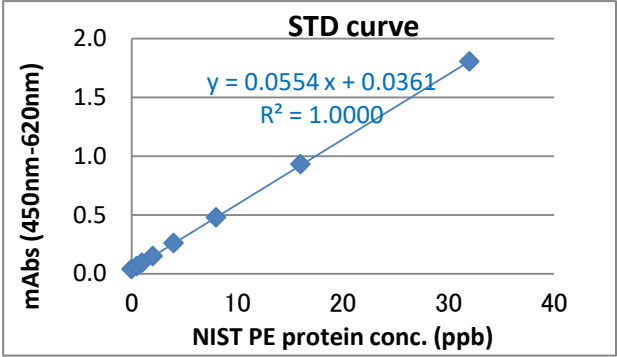
4. Analytical Sensitivity

4.1

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.

Standard curve: mAbs (450nm / 620nm)

STD (ppb)	Ave.	CV%
0	0.036	4.7
0.5	0.062	3.7
1	0.090	3.2
2	0.147	2.6
4	0.259	2.7
8	0.477	3.1
16	0.931	2.2
32	1.806	1.9



Blank	0 ppm A	0 ppm B
1	0.007	0.000
2	0.000	0.022
3	0.007	0.000
4	0.014	0.000
5	0.000	0.000
6	0.007	0.000
7	0.000	0.000
8	0.007	0.000
Ave.	0.004	
SD	0.006	

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

*Calculated by linear regression

LOD	0.023 ppm
LOQ	0.068 ppm

Reversed quant value +3SD
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 Method

Primary Food Matrices	Water	Chocolate	Cookie	Ice Cream	Sauce	Dressing
1	0.01	(-)	(-)	0.05	(-)	0.03
2	0.02	(-)	0.00	0.05	(-)	0.01
3	0.02	(-)	0.00	0.07	(-)	(-)
4	0.02	(-)	0.03	0.04	(-)	0.02
5	0.02	(-)	0.02	(-)	(-)	0.01
6	0.02	(-)	0.02	(-)	(-)	0.01
7	0.01	(-)	0.00	(-)	(-)	0.01
8	0.01	(-)	0.00	0.01	(-)	0.02
9	0.02	(-)	0.01	0.05	(-)	0.03
10	0.02	(-)	0.05	(-)	(-)	0.01
Mean, ppm	0.02	<0.01	0.02	0.04	<0.01	0.02
SD	0.01	-	0.02	0.02	-	0.01
Limit of Detection, ppm	0.03	<0.01	0.06	0.10	<0.01	0.04
Limit of Quantification, ppm	0.07	<0.01	0.18	0.24	<0.01	0.10

Extraction : Short time Extraction Method

Primary Food Matrices	Water	Chocolate	Cookie	Ice Cream	Sauce	Dressing
1	0.01	(-)	0.05	0.04	(-)	(-)
2	0.03	(-)	0.03	0.01	(-)	0.01
3	0.03	0.00	0.07	(-)	(-)	0.01
4	0.02	(-)	0.04	(-)	(-)	0.02
5	0.00	(-)	0.01	(-)	(-)	0.02
6	0.02	(-)	0.03	0.01	(-)	0.02
7	0.02	(-)	(-)	0.01	(-)	0.03
8	0.02	0.03	0.00	0.00	(-)	0.01
9	0.02	(-)	0.02	0.01	(-)	(-)
10	0.03	(-)	0.05	0.04	(-)	0.01
Mean	0.02	0.02	0.03	0.02	<0.01	0.02
SD	0.01	0.02	0.02	0.02	-	0.01
Limit of Detection, ppm	0.05	0.07	0.10	0.06	<0.01	0.04
Limit of Quantification, ppm	0.11	0.20	0.24	0.17	<0.01	0.10

5. Cross-Reactivity

Cross-reactivity (results<LOQ) of variety foods were confirmed.

Unit: µg NIST peanut butter protein/g food

Extraction: Overnight method

Please visit http://www.miobs-e.com/product/food_allergen_elisa2/data/index.html for the latest data sheet.

6. Criteria for the standard curve

In preparation