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Validation Report

Hazelnut ELISA Kit II (Cat.# M2119)

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

Limit of Detection: 0.16 µg hazelnut protein/g food

Standard Range: 0.16-10 µg hazelnut protein/g food

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1. Scope

The Hazelnut ELISA Kit II is sandwich enzyme immunoassay for the quantitative determination of hazelnut 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 : Short Time Extraction Method

Replicate	control 1	control 2	control 3
1	7.18	9.78	10.67
2	7.23	9.50	10.38
3	7.34	10.05	10.40
Mean	7.25	9.78	10.48
SD	0.08	0.27	0.17
CV(%)	1.1%	2.8%	1.6%

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 : Short Time Extraction Method

Assay No.	control 1	control 2	control 3
1	7.19	9.61	10.40
2	7.23	9.50	10.38
3	7.18	9.78	10.67
Mean	7.20	9.63	10.48
SD	0.03	0.14	0.17
CV(%)	0.4%	1.5%	1.6%

3. Recovery

3.1 Incurred foods

For recovery experiments,

hazelnut incurred foods were prepared with 10ppm protein of hazelnut contamination.

Regarding the spiking foods, 5 ppm hazelnut protein were added.

Samples		Overnight Extraction		Short time extraction	
		Conc. [ppm]	Recovery	Conc. [ppm]	Recovery [※]
Incurred foods	Ice cream	7.72	80%	10.22	105%
	Cookie	6.90	68%	7.57	75%
	Bread	7.47	75%	9.19	92%
	Cocoa cake	6.98	70%	10.64	106%
Spiked foods	Almond (roasted)	NT		3.2	64%
	Macadamia (roasted)	NT		5.1	101%
	Cashew (roasted)	NT		3.0	61%
	Pistachio (roasted)	NT		4.9	97%
	Brazil nut	NT		3.4	69%
	Lupin beans	NT		2.7	55%
Hazelnut (roasted) x200 dilution		10958.0	82%	12850.1	96%
Hazelnut (raw) x200 dilution		10740.4	81%	12338.7	93%

※The recovery of spiked foods were calculated with 5 ppm of theoretical value.

Measured value by Kjeldahl method

Hazelnut (roasted)	13.4 g/100g	13400 ppm
Hazelnut (raw)	13.3 g/100g	13300 ppm

3.2 Spiked foods

For recovery experiments different sample matrices were spiked with Hazelnut powder.
The contamination levels were 0.5, 1, 2.5, 5 ppm.

Each extraction option of the Hazelnut 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 (%)
5.0	4.0	80%
2.5	2.1	82%
1.0	0.9	95%
0.5	0.5	100%
	Mean	89%

Short Time Extraction Method

Water

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	4.3	87%
2.5	2.3	93%
1.0	1.0	101%
0.5	0.5	100%
	Mean	95%

White pepper

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	1.6	33%
2.5	0.9	36%
1.0	0.4	36%
0.5	0.2	40%
	Mean	36%

White pepper

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	5.2	103%
2.5	2.6	102%
1.0	1.1	110%
0.5	0.6	113%
	Mean	107%

Dressing

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	4.1	82%
2.5	2.3	90%
1.0	1.0	97%
0.5	0.5	99%
	Mean	92%

Dressing

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	4.4	89%
2.5	2.5	98%
1.0	1.1	106%
0.5	0.5	108%
	Mean	100%

Cookie

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	2.4	47%
2.5	1.4	55%
1.0	0.7	71%
0.5	0.4	76%
	Mean	62%

Cookie

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	5.6	112%
2.5	2.8	113%
1.0	1.2	125%
0.5	0.6	129%
	Mean	120%

Ice Cream

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	3.5	71%
2.5	2.0	78%
1.0	0.9	87%
0.5	0.5	92%
	Mean	82%

Ice Cream

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	3.6	72%
2.5	1.7	70%
1.0	0.7	74%
0.5	0.4	73%
	Mean	72%

Pasta

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	1.8	36%
2.5	1.2	47%
1.0	0.5	46%
0.5	0.3	54%
	Mean	46%

Pasta

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	3.5	70%
2.5	1.8	71%
1.0	0.6	59%
0.5	0.4	77%
	Mean	69%

Chocolate

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	2.5	50%
2.5	1.3	52%
1.0	0.5	55%
0.5	0.3	54%
	Mean	53%

Chocolate

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	4.8	96%
2.5	2.5	102%
1.0	1.1	107%
0.5	0.6	110%
	Mean	104%

4. Analytical Sensitivity

4.1

For determination of the analytical sensitivity, sample diluent was assayed in 3-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.

STD [ng/mL]	OD			
	1	2	3	Ave.
0	0.011	0.01	0.01	0.010
0.39	0.031	0.029	0.029	0.030
0.78	0.057	0.052	0.051	0.053
1.56	0.107	0.104	0.102	0.104
3.13	0.214	0.202	0.199	0.205
6.25	0.433	0.416	0.418	0.422
12.50	0.918	0.877	0.863	0.886
25.00	1.896	1.861	1.854	1.870

⇒ LOD, LOQ

◆ Calculation of LOD · LOQ

Blank	0.010
SD	0.001

LOD (3SD)	0.012
LOQ (10SD)	0.016

LOD = Blank + 3SD

LOQ = Blank + 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	White pepper	Dressing	Cookie	Ice Cream	Pasta	Chocolate
1	0.00	0.00	0.00	0.04	0.00	0.00	0.00
2	0.00	0.00	0.00	0.01	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.01	0.02	0.00	0.00	0.00
5	0.00	0.01	0.08	0.00	0.00	0.00	0.00
6	0.00	0.00	0.01	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.01	0.00	0.00	0.00
8	0.00	0.02	0.00	0.00	0.00	0.00	0.00
9	0.00	0.01	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean, ppm	0.00	0.00	0.01	0.01	0.00	0.00	0.00
SD	0.00	0.01	0.03	0.01	0.00	0.00	0.00
Limit of Detection, ppm	<0.01	0.02	0.08	0.05	<0.01	<0.01	<0.01
Limit of Quantification, ppm	<0.01	0.06	0.26	0.15	<0.01	<0.01	<0.01

Extraction : Short time Extraction Method

Primary Food Matrices	Water	White pepper	Dressing	Cookie	Ice Cream	Pasta	Chocolate
1	0.00	0.00	0.01	0.00	0.00	0.00	0.00
2	0.00	0.00	0.02	0.00	0.01	0.00	0.00
3	0.00	0.00	0.06	0.00	0.00	0.00	0.00
4	0.00	0.00	0.03	0.00	0.00	0.00	0.00
5	0.00	0.00	0.02	0.00	0.00	0.00	0.00
6	0.00	0.00	0.02	0.00	0.00	0.00	0.00
7	0.00	0.03	0.02	0.01	0.00	0.00	0.00
8	0.00	0.00	0.01	0.00	0.01	0.00	0.00
9	0.00	0.01	0.02	0.00	0.00	0.00	0.00
10	0.00	0.00	0.07	0.00	0.00	0.00	0.00
Mean	0.00	0.00	0.03	0.00	0.00	0.00	0.00
SD	0.00	0.01	0.02	0.00	0.00	0.00	0.00
Limit of Detection, ppm	<0.01	0.03	0.09	0.01	0.01	<0.01	<0.01
Limit of Quantification, ppm	<0.01	0.09	0.23	0.02	0.04	<0.01	<0.01

5. Cross-Reactivity

For the following foods, each cross-reactivity (results<LOQ) were confirmed.

Unit: µg hazelnut protein/g food

Extraction: Overnight method

Rice powder	< 0.16	Almond (roasted)	< 0.16	Lupin beans	< 0.16
Buckwheat	< 0.16	Macadamia (roasted)	< 0.16	Rye	< 0.16
Wheat	< 0.16	Cashew (roasted)	< 0.16	Oat	< 0.16
Soy	< 0.16	Pistachio (roasted)	< 0.16	Cumin	< 0.16
Peanut	< 0.16	Walnut (roasted)	< 0.16	Coriander	< 0.16
Shrimp	< 0.16	Peacan nut (roasted)	< 0.16	Black pepper	< 0.16
Crub	< 0.16	Almond (raw)	< 0.16	White pepper	< 0.16
Egg	< 0.16	Macadamia (raw)	< 0.16	Turmeric	< 0.16
Egg (boiled)	< 0.16	Cashew (raw)	< 0.16	Basil	< 0.16
Dried Egg	< 0.16	Pistachio (raw)	< 0.16	Red pepper	< 0.16
Milk	< 0.16	Walnut (raw)	< 0.16	Bell pepper	< 0.16
Skim milk	< 0.16	Peacan nut (raw)	< 0.16		
Corn flour	< 0.16	Brazil nut	< 0.16		

Extraction : Short time Extraction Method

Rice powder	< 0.16	Almond (roasted)	< 0.16	Lupin beans	< 0.16
Buckwheat	< 0.16	Macadamia (roasted)	< 0.16	Rye	< 0.16
Wheat	< 0.16	Cashew (roasted)	< 0.16	Oat	< 0.16
Soy	0.22	Pistachio (roasted)	< 0.16	Cumin	< 0.16
Peanut	< 0.16	Walnut (roasted)	< 0.16	Coriander	< 0.16
Shrimp	< 0.16	Peacan nut (roasted)	< 0.16	Black pepper	< 0.16
Crub	< 0.16	Almond (raw)	< 0.16	White pepper	< 0.16
Egg	< 0.16	Macadamia (raw)	< 0.16	Turmeric	< 0.16
Egg (boiled)	< 0.16	Cashew (raw)	< 0.16	Basil	< 0.16
Dried Egg	< 0.16	Pistachio (raw)	< 0.16	Red pepper	< 0.16
Milk	< 0.16	Walnut (raw)	< 0.16	Bell pepper	< 0.16
Skim milk	< 0.16	Peacan nut (raw)	< 0.16		
Corn flour	< 0.16	Brazil nut	< 0.16		

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

	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°C.

※2 R² value by using 4-parameter analysis on ELISA data.

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