Date of issue: August 14th 2019

Validation Report

Crustacean ELISA Kit II (Cat.# M2118)

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

Limit of Detection：**0.31 μg crustacean protein/g food**

Standard Range：**0.31-20 μg crustacean protein/g food**

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1. Scope

The **Crustacean ELISA Kit Ⅱ** is sandwich enzyme immunoassay for the quantitative determination of crustacean 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	9.95	12.98	8.16	5.43
2	10.01	12.98	8.14	5.54
3	10.69	13.32	8.52	5.81
Mean	10.22	13.09	8.27	5.59
SD	0.41	0.19	0.22	0.20
CV%	4.1%	1.5%	2.6%	3.5%

Extraction : Short Time Extraction Method

Assay No.	control 1	control 2	control 3	control 4
1	9.99	13.20	7.74	6.15
2	10.01	13.23	7.92	6.18
3	10.47	13.49	8.00	6.28
Mean	10.15	13.30	7.89	6.20
SD	0.27	0.16	0.13	0.07
CV%	2.7%	1.2%	1.7%	1.1%

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 : Short Time Extraction Method

Assay No.	control 1	control 2	control 3	control 4
day 1	12.8	7.4	6.2	9.7
day 2	12.9	8.4	6.7	10.6
day 3	13.3	8.1	6.6	10.0
Mean	13.0	8.0	6.5	10.1
SD	0.3	0.5	0.3	0.4
CV%	2.0%	6.1%	4.2%	4.2%

3. Recovery

3.1 Incurred foods

For recovery experiments,
crustacean incurred foods were prepared with 10ppm protein of crustacean contamination.

Extraction : Overnight Extraction Method

Food samples	Heating condition	Actual Concentration [ppm]	Recovery [%]
Chicken meat ball	None heat treatment	8.2	82%
Tomato sauce	Heated at 121℃ for 20min	10.3	103%
Fish sausage	Steamed at 90℃ for 15min	13.3	133%
Jelly	Heated up to 95℃ for 1min	7.0	70%
Bread	Baked by home bakery machine	5.8	58%
Corn soup	Heated at 95℃ for 10min	9.8	98%
Bean soup	Heated at 95℃ for 10min	9.8	98%
Fish cake	Steamed at 90℃ for 15min	9.8	98%

Extraction : Short Time Extraction Method

Food samples	Heating condition	Actual Concentration [ppm]	Recovery [%]
Chicken meat ball	None heat treatment	10.7	107%
Tomato sauce	Heated at 121℃ for 20min	8.7	87%
Fish sausage	Steamed at 90℃ for 15min	13.7	137%
Jelly	Heated up to 95℃ for 1min	6.6	66%
Bread	Baked by home bakery machine	5.9	59%
Corn soup	Heated at 95℃ for 10min	10.1	101%
Bean soup	Heated at 95℃ for 10min	9.4	94%
Fish cake	Steamed at 90℃ for 15min	10.9	109%

3.2 Spiked foods

For recovery experiments different sample matrices were spiked with crustacean powder material (original). The contamination levels were 0.5, 1, 2.5, 5. Each extraction option of the Crustacean ELISA Kit Ⅱ was tested by 3 individual extracted samples per contamination level.

Extraction : Overnight Extraction Method & Short Time Extraction Method

Overnight Extraction Method

Water

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	4.9	97%
2.5	2.4	97%
1.0	1.0	102%
0.5	0.5	102%
	Mean	100%

Sauce

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	5.2	104%
2.5	2.6	104%
1.0	1.1	108%
0.5	0.5	97%
	Mean	103%

Surimi

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	5.6	112%
2.5	2.7	108%
1.0	1.1	111%
0.5	0.6	114%
	Mean	111%

Short Time Extraction Method

Water

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	4.7	95%
2.5	2.4	96%
1.0	1.0	101%
0.5	0.5	105%
	Mean	99%

Sauce

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	5.1	101%
2.5	2.6	102%
1.0	1.1	107%
0.5	0.5	105%
	Mean	104%

Surimi

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	6.0	120%
2.5	2.9	118%
1.0	1.2	122%
0.5	0.6	125%
	Mean	121%

Soup

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	5.1	101%
2.5	2.3	93%
1.0	1.0	97%
0.5	0.5	94%
	Mean	96%

Soup

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	4.7	95%
2.5	2.4	96%
1.0	1.0	102%
0.5	0.5	98%
	Mean	98%

Snack

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	5.8	116%
2.5	2.9	114%
1.0	1.1	114%
0.5	0.5	109%
	Mean	113%

Snack

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
5.0	5.9	118%
2.5	3.1	122%
1.0	1.2	125%
0.5	0.6	119%
Mean		121%

4. Analytical Sensitivity

4.1

For determination of the analytical sensitivity, sample diluent was 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.
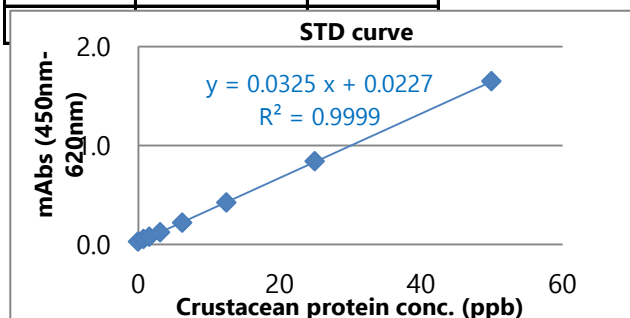
Standard curve: mAbs (450nm / 620nm)

STD (ppb)	Ave.	CV%
0	0.027	3.5%
0.78	0.053	5.3%
1.56	0.076	3.7%
3.12	0.122	1.7%
6.25	0.218	2.3%
12.5	0.422	4.2%
25	0.839	0.3%

Blank	mAbs (450/620nm)	Reversed quant value (ppb)
1	0.029	0.19
2	0.028	0.16
3	0.026	0.10
4	0.028	0.16
5	0.028	0.16
6	0.027	0.13
7	0.026	0.10
8	0.027	0.13
9	0.027	0.13
10	0.028	0.16
Ave	0.027	0.145
SD	0.001	0.030

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

*Calculated by linear regression

STD curve

$y = 0.0325 x + 0.0227$
$R^2 = 0.9999$

mAbs (450nm-620nm)

Crustacean protein conc. (ppb)

LOD	0.18	ppm	Reversed quant value +3SD
LOQ	0.26	ppm	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 devation was defined as limit of detection and OD mean + 10 x standard devation was defined as limit of quantification.

Extraction : Overnight Extraction Method *Calculated by linear regression

Primary Food Matrices	Water	Sauce	Surimi	Soup	Snack
1	0.06	0.07	0.04	0.11	0.10
2	0.07	0.04	0.09	0.05	0.12
3	0.08	0.01	0.06	0.00	0.11
4	0.07	0.07	0.10	0.13	0.16
5	0.06	0.04	0.06	0.06	0.12
6	0.05	0.08	0.03	0.09	0.09
7	0.03	0.07	0.02	0.09	0.05
8	0.06	0.09	0.06	0.10	0.18
9	0.06	0.06	0.04	0.09	0.10
10	0.07	0.06	0.10	0.05	0.10
Mean, ppm	0.06	0.06	0.06	0.08	0.11
SD	0.01	0.02	0.03	0.04	0.04
Limit of Detection, ppm	0.10	0.13	0.14	0.19	0.22
Limit of Quantification, ppm	0.20	0.29	0.33	0.46	0.47

Extraction : Short time Extraction Method *Calculated by linear regression

Primary Food Matrices	Water	Sauce	Surimi	Soup	Snack
1	0.06	0.04	0.07	0.02	0.10
2	0.08	0.10	0.12	0.11	0.11
3	0.05	0.08	0.08	0.06	0.10
4	0.05	0.06	0.03	0.06	0.11
5	0.02	0.06	0.01	0.06	0.09
6	0.09	0.06	0.10	0.06	0.14
7	0.06	0.06	0.04	0.06	0.12
8	0.08	0.05	0.08	0.06	0.13
9	0.06	0.03	0.07	0.05	0.13
10	0.10	0.15	0.12	0.08	0.14
Mean, ppm	0.06	0.07	0.07	0.06	0.12
SD	0.02	0.04	0.04	0.02	0.02
Limit of Detection, ppm	0.13	0.18	0.18	0.13	0.17
Limit of Quantification, ppm	0.28	0.42	0.45	0.28	0.29

5. Cross-Reactivity

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

Unit：μg curatacean protein/g food

5.1 Sea foods

Sample	Extraction method	Value [μg/g]	Sample	Extraction method	Value [μg/g]
Surf clam	Overnight	-	Squid	Overnight	0.42
Arch shell	Overnight	0.40	Sepia officinalis	Overnight	-
Clam	Overnight	-	Okhotsk atka mackerel	Overnight	-
White whelk	Overnight	1.29	Pacific whiting	Overnight	-
Firefly squid	Overnight	1.01	Salmon roe	Overnight	-
Lipped mussel	Overnight	-	Pollack roe	Overnight	-
Octopus ocellatus	Overnight	-	Pork meat	Overnight	-
Asari clam	Overnight	-	Beef meat	Overnight	-
Salmon	Overnight	-	Scallop	Overnight	-
Pollack	Overnight	-	Blue mussel	Overnight	-
Golden threadfin bream	Overnight	-	Octopus dofleini	Overnight	-
Octopus vulgaris	Overnight	-	Spear squid	Overnight	-
Flying fish	Overnight	-	Scallop	Short time Ex.	-
Scabbard fish	Overnight	-	Blue mussel	Short time Ex.	-
Croaker fish	Overnight	-	Octopus dofleini	Short time Ex.	-
Lizardfish	Overnight	-	Spear squid	Short time Ex.	-

Short time Ex. = Short time extraction

5.2 Shrimp and Crabs

Sample	Extraction method	Reactivity rate *	ELISA value [mg/mL]	Reference value [mg/mL]**	E/R***
Black tiger shrimp	Overnight	100%	1.36	1.84	73.7%
Sweet shrimp	Overnight	109%	1.26	1.98	63.7%
Spiny lobster	Overnight	76%	2.35	2.09	112.4%
Lobster	Overnight	72%	1.27	-	-
Red king crab	Overnight	60%	1.09	1.3	84.0%
Snow crab	Overnight	127%	1.08	1.39	77.5%
Blue crab	Overnight	103%	1.22	1.44	84.7%
Hair crab	Overnight	87%	1.54	1.58	97.3%

* Calculated the black tiger shrimp as 100%   *** ELISA Value [mg/mL] / Reference value [mg/mL]

** Reference value was used from the food composition table provided by Ministry of Education, Culture, Sports, Science and Technology

5.3 Various foods

Please visit **http://www.miobs-e.com/product/food_allergen_elisa2/dat** 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℃.

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

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