

Validation Report

Buckwheat ELISA Kit II

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

Limit of Detection: 0.31 μ g buckwheat protein/g food

Standard Range: 0.31–20 μ g buckwheat protein/g food

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1. Scope

The **Buckwheat ELISA Kit II** is sandwich enzyme immunoassay for the quantitative determination of buckwheat 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	9.40	8.42	7.27
2	9.52	7.41	7.72
3	9.45	6.70	7.08
4	9.98	8.34	7.67
5	8.90	7.37	7.36
Mean	9.45	7.65	7.42
SD	0.38	0.73	0.27
CV(%)	4.1%	9.5%	3.7%

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	10.86	8.54	7.15
2	9.52	8.34	7.72
3	10.83	9.06	7.34
Mean	10.40	8.65	7.40
SD	0.77	0.37	0.29
CV(%)	7.4%	4.3%	3.9%

3. Recovery

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

Extraction : Overnight Extraction Method

Food samples	Heating condition	Actual Concentration (ppm)	Recovery (%)
Orange Juice	Heated at 90°C for 10min	12.0	120%
Biscuit	Heated 8.5min by oven	6.7	67%

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 deviation was defined as limit of detection and OD mean + 10 x standard deviation was defined as limit of quantification.

Replicate	0ng/mL(OD)
1	0.010
2	0.012
3	0.013
4	0.011
Mean	0.012
SD	0.001
Limit of Detection	0.31µg buckwheat protein/g food
Limit of Quantification	0.31µg buckwheat protein/g food

5. Cross-Reactivity

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

Unit: µg buckwheat protein/g food

Egg	<0.31
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)	0.48
Cashew (Roasted)	<0.31
Macadamia (Roasted)	<0.31
Pistachio (Roasted)	0.33
Walnut(Roasted)	<0.31
Sesame(Roasted)	1.67
Black pepper	<0.31
Red pepper	1.38
Cumin	<0.31
Coriander	0.67
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/B ₀ (= 50ng/mL absorbance value / blank absorbance value)	≥ 10

※1 The incubation temperature of ELISA reaction is all 25°C.

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

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