

Validation Report

Peanut ELISA Kit II

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

Limit of Detection: 0.31µg peanut protein/g food

Standard Range: 0.31–20 µg peanut protein/g food

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1. Scope

The **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 three controls in 5-fold replicates.
Extraction : Overnight Extraction Method

Replicate	control 1	control 2	control 3
1	8.89	9.38	7.58
2	8.90	9.72	7.69
3	8.56	8.86	7.08
4	8.45	9.18	7.59
5	8.47	9.09	7.38
Mean	8.65	9.25	7.46
SD	0.22	0.32	0.24
CV(%)	2.6%	3.5%	3.2%

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	8.76	9.79	7.99
2	9.34	10.36	7.94
3	8.89	9.38	7.58
Mean	9.00	9.84	7.84
SD	0.30	0.49	0.22
CV(%)	3.4%	5.0%	2.9%

3. Recovery

For recovery experiments, peanut incurred foods were prepared with 10ppm protein of peanut contamination.
Extraction : Overnight Extraction Method

Food samples	Heating condition	Actual Concentration (ppm)	Recovery (%)
Orange Juice	Heated at 90°C for 10min	6.3	63%
Jelly	Heated up to reach 90°C	9.4	94%
Cake	Heated for 2min in a microwave oven	11.0	110%
Porridge	Cooked by rice cooker	8.5	85%

For another experiment, two sample matrices were spiked with peanut to obtain various final concentrations before extraction.
Extraction : Overnight Extraction Method

Jelly

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
10	8.5	85%
5	4.0	80%
1	0.9	90%

Cooked rice

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
10	7.7	77%
5	4.9	98%
1	1.2	120%

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	0.029
2	0.027
3	0.026
4	0.026
Mean	0.027
SD	0.001
Limit of Detection	0.31µg peanut protein/g food
Limit of Quantification	0.31µg peanut protein/g food

5. Cross-Reactivity

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

Unit: µg peanut 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	>20
Almond (Roasted)	<0.31
Cashew (Roasted)	<0.31
Macadamia (Roasted)	<0.31
Pistachio (Roasted)	<0.31
Walnut(Roasted)	<0.31
Sesame(Roasted)	<0.31
Black pepper	<0.31
Red pepper	<0.31
Cumin	<0.31
Coriander	<0.31
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/B0 (= 50ng/mL absorbance value / blank absorbance value)	≥10

×1 The incubation temperature of ELISA is all 25°C.
×2 R^2 value by using 4–parameter analysis on ELISA data.
4–Parameter fit: Y=(A–D)/(1+(X/C)^B)+D