Validation Report

Soya ELISA Kit Ⅱ

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

Limit of Detection: 0.31μg soya protein/g food

Standard Range: 0.31-20 μg soya protein/g food

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1. Scope

The **Soya ELISA Kit Ⅱ** is sandwich enzyme immunoassay for the quantitative determination of soya 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 : Overnight Extraction Method

Replicate	control 1	control 2	control 3
1	8.48	11.92	6.61
2	8.68	11.97	6.55
3	8.46	11.73	6.56
Mean	8.54	11.87	6.57
SD	0.12	0.13	0.03
CV(%)	1.4%	1.1%	0.5%

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	8.79	11.94	6.55
2	8.48	11.92	6.61
3	8.37	11.38	6.30
Mean	8.55	11.75	6.49
SD	0.22	0.32	0.16
CV(%)	2.5%	2.7%	2.5%

3. Recovery

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

Food samples	Heating condition	Actual Concentration (ppm)	Recovery (%)
Bread	Approximately, Heated at 180-200℃ for 40min (Cooked bread baking machine)	7.8	78%
Jelly	Heated up to reach 90℃	12.2	122%
Fish sausage	Heated at 100℃ for 20 min	7.7	77%

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

Replicate	0ng/mL(OD)
1	0.039
2	0.039
3	0.042
4	0.038
Mean	0.040
SD	0.002
Limit of Detection	0.31µg soya protein/g food
Limit of Quantification	0.31µg soya protein/g food

5. Cross-Reactivity

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

Unit: µg soya 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	＞20
Corn flour	＜0.31
Peanut	＜0.31
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	0.44
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℃.
※2 R^2 value by using 4-parameter analysis on ELISA data.
4-Parameter fit: Y=(A-D)/(1+(X/C)^B)+D