Validation Report

Wheat/Gluten (Gliadin) ELISA Kit II

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

Limit of Detection: 0.31μg wheat protein/g food

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

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1. Scope

The **Wheat/Gluten (Gliadin) ELISA Kit II** is sandwich enzyme immunoassay for the quantitative determination of wheat 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	8.72	9.60	9.70
2	8.84	9.96	10.28
3	8.61	9.62	10.38
4	8.93	9.99	10.40
5	9.02	9.51	9.51
Mean	8.82	9.74	10.05
SD	0.16	0.22	0.42
CV(%)	1.8%	2.3%	4.2%

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	9.10	9.30	11.23
2	9.08	9.27	10.72
3	8.41	8.55	10.52
Mean	8.86	9.04	10.82
SD	0.39	0.42	0.37
CV(%)	4.4%	4.7%	3.4%

3. Recovery

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

Food samples	Heating condition	Actual Concentration (ppm)	Recovery (%)
Orange Juice	Heated at 90℃ for 10min	9.8	98%
Jelly	Heated up to reach 90℃	8.7	87%
Cake	Heated for 2min in a microwave oven	11.8	118%
Porridge	Cooked by rice cooker	10.8	108%

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

Jelly

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
10	8.7	87%
5	4.3	86%
1	0.8	80%

Cooked rice

Target Value (ppm)	Actual Concentration (ppm)	Recovery (%)
10	11.0	110%
5	5.7	114%
1	1.3	130%

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	0.021
2	0.020
3	0.022
4	0.018
Mean	0.020
SD	0.002
Limit of Detection	0.31μg wheat protein/g food
Limit of Quantification	0.31μg wheat protein/g food

5. Cross-Reactivity

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

Unit: μg wheat protein/g food

Egg	＜0.31
Milk	＜0.31
Skim milk	＜0.31
Wheat	＞20
Barley	＞20
Rye	＞20
Oats	＞20
Soy bean	＜0.31
Corn flour	＜0.31
Peanut	＜0.31
Almond (Roasted)	2.36
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	0.52
Cumin	1.36
Coriander	1.13
Poppy seed	1.08
Shrimp	＜0.31
Crab	0.62
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