



V.7/Nov/2021

Wheat/Gluten ELISA Kit

For the Quantitative Determination of
Wheat Protein/Gluten in Food

Catalog #M2103
96 Assays



For Research or Laboratory Use Only.
Not for Use in Diagnostic Procedures.
Please read full descriptions in this manual before use.

*** Previous Name : Wheat/Gluten (Gliadin) ELISA Kit**



E-mail: info_miobs_e@morinaga.co.jp

Website: <https://www.miobs-e.com>

JP.PAT.3600231

TABLE OF CONTENTS

INTENDED USE AND USER.....	3
PRINCIPLE OF ELISA.....	3
GENERAL INFORMATION.....	4
APPROVAL/VALIDATION.....	4
KIT COMPONENTS.....	4
REQUIRED MATERIALS (NOT INCLUDED).....	5
PERFORMANCE CHARACTERISTICS.....	6
REAGENT PREPARATION.....	7
LOW RANGE ASSAY PROTOCOL	
(0-20 ppm wheat protein, 0-17 ppm gluten).....	8
<i>Results and Interpretation</i>	11
<i>Typical Standard Curve</i>	11
<i>Conversion to gluten</i>	12
<i>Summary of Procedure</i>	12
HIGH RANGE ASSAY PROTOCOL (0-68 ppm gluten)	14
<i>Results and Interpretation</i>	17
<i>Typical Standard Curve</i>	17
<i>Summary of Procedure</i>	18
STORAGE CONDITIONS AND EXPIRY OF KIT.....	19
WARNINGS AND PRECAUTIONS.....	19
<i>Reagent preparation</i>	21
REFERENCE.....	22
TECHNICAL ASSISTANCE.....	22
WARRANTY.....	23

For optimal results, follow instructions exactly. Failure to do so may lead to inaccurate results.

Reproducible results depend on careful pipetting technique, maintaining incubations at the specified temperature for the specified time, complete washing, and thorough mixing of all solutions.

INTENDED USE AND USER

The Wheat /Gluten ELISA kit was designed to quantify total wheat protein in raw materials and processed foods with cross-reactivity to barley and rye protein and, using a conversion factor (0.85), convert the wheat, barley and rye protein to the gluten content (*see page 12, CONVERSION TO GLUTEN*). This kit provides two options for sample extraction and two ELISA assay methods. Each extraction and ELISA assay method is available for both wheat protein and gluten analysis, but it is recommended to select the extraction and ELISA assay method for use as follows:

1. Overnight extraction and low range assay: to quantify total wheat protein in raw materials and finished products, including processed foods for allergen analysis.
2. Short time extraction and low range assay: to quantify total wheat protein in raw materials and finished products, including processed foods for allergen analysis where the results are required the same day.
3. Overnight extraction and high range assay: to quantify the gluten content in raw materials and gluten free products, including processed foods.
4. Short time extraction and high range assay: to quantify the gluten content in raw materials and gluten free products, including processed foods for gluten analysis where the results are required the same day.

PRINCIPLE OF ELISA

The Wheat/Gluten ELISA kit is a sandwich enzyme linked immunosorbent assay based on anti-gliadin polyclonal antibody. Gliadin in food samples is extracted by heating in boiling water for 10 minutes or shaking overnight with an extraction buffer, which includes a detergent and reducing agent. The complex of antibody, antigen, and enzyme-conjugated antibody is formed by a two-step reaction and is accompanied by color development. Absorbance is measured at wavelength 450 nm, after the addition of stop solution. The concentration of wheat protein/gluten corresponding to the measured absorbance can be determined by preparing a wheat protein standard solution curve.

GENERAL INFORMATION

Wheat allergy is a specific immune reaction to various wheat proteins, including albumin, globulin, gliadin, and glutenin. Wheat has been considered a common allergen for a long time, for example, the occupational hazard of baker's asthma has been reported over many years. In addition, food dependent exercise-induced anaphylaxis (FDEIA) can occur in affected individuals if they eat wheat before exercise. Persons with a wheat allergy need to avoid eating food containing wheat protein, therefore, it is very important that the presence of wheat is declared on food labels.

Gluten is a type of protein found in grains, such as wheat, barley, and rye. It is responsible for the induction of some autoimmune diseases, commonly referred to as gluten insensitivity or celiac disease. In autoimmune diseases caused by gluten, the affected individual cannot digest gluten and this causes issues such as inflammation of the surface of the small intestine, diarrhea, and vomiting. There is no treatment; therefore, it is very important for affected individuals to eat foods that are classified as "gluten-free" as a symptomatic treatment.

Food manufacturers are required to declare the presence of wheat/gluten on food packaging, therefore it is essential that naturally occurring or contaminated sources of wheat/gluten can be easily detected.

APPROVAL/VALIDATION

1. AOAC Research Institute *Performance Tested Method*SM #011804.
2. Official Japanese allergen (wheat protein) analysis method (LOW RANGE ASSAY and Overnight Extraction Method)
3. Gluten analysis method, cited in the gluten free labeling rules in the USA as the gluten determination method (Federal Register Vol.78, No.150, P47154)

KIT COMPONENTS

Label	Name of component	Content	Quantity
A	Antibody-coated Microplate Module	6X8 well modules	2 packs
B	Wheat Protein Standard (50 ppb)	1 mL	2 vials
C	Enzyme-conjugated Antibody	13 mL	1 bottle
D	Enzyme Substrate (TMB Solution)	13 mL	1 bottle
E	Stop Solution (1N Sulfuric Acid)	13 mL	1 bottle
F	Sample Buffer (20X Concentrate)	50 mL	1 bottle
G	Wash Buffer (20X Concentrate)	50 mL	1 bottle
H	Extraction Component A (20X Concentrate)	55 mL	1 bottle
	Frame for mounting the microplate module		1 piece
	Microplate cover		1 piece

REQUIRED MATERIALS (NOT INCLUDED)

1. 2-Mercaptoethanol
2. Distilled water (or deionized water)
3. Micropipettes and disposable tips ranging from 20 to 1000 μ L
4. Graduated cylinders
5. Polypropylene centrifuge tubes (50 mL size) for sample extraction
6. Polypropylene micro tubes (1–2 mL size) for preparation of working standard solutions and dilution of samples
7. Tube racks
8. pH test paper
9. Homogenizer/blender for sample preparation (if necessary)
10. Water bath for boiling or horizontal shaker, for sample extraction
11. Centrifuge
12. Vortex mixer
13. Aspirator for washing procedure, or optional microplate washer
14. Microplate reader with a 450 nm filter, and a filter for wavelengths from 600 to 650 nm

PERFORMANCE CHARACTERISTICS

The Wheat/Gluten ELISA kit is a tool to determine the presence of wheat protein in raw and processed foods.

Sample preparation time	Approx. 10 min	
Sample extraction time	Approx. 30 min, or overnight	
Time taken for ELISA	Approx. 2 h	
Assay sensitivity	Low Range Assay	0.31 ppm wheat protein (0.26 ppm gluten*)
	High Range Assay	1.05 ppm gluten*
Detectable concentration range	0.78 to 50 ppb wheat protein	
Limit of detection (no matrix)	Short time extraction and low range assay	0.05 ppm
	Overnight extraction and low range assay	0.08 ppm
	Short time extraction and high range assay	0.13 ppm
	Overnight extraction and high range assay	0.03 ppm
Limit of quantitation (no matrix)	Short time extraction and low range assay	0.16 ppm
	Overnight extraction and low range assay	0.25 ppm
	Short time extraction and high range assay	0.38 ppm
	Overnight extraction and high range assay	0.09 ppm
Validated matrixes	AOAC Research Institute <i>Performance Tested Method</i> ^{6M}	Cider, gluten free bread, ice cream, rice flour, soup, and water
	Official Japanese allergen analysis method (wheat protein)	Steamed fish paste, orange juice, custard pudding, baby food (Japanese-style mixed stew cooked in a retort pouch), and tomato sauce

*See ***Conversion to Gluten***.

Additional specificity data are available, if required, please contact Morinaga Institute of Biological Science, Inc., or your local distributor.

REAGENT PREPARATION

Sample Extraction Solution

Prepare the **Sample Extraction Solution** by mixing **Sample Buffer (F)**, **Extraction Component A (H)**, 2-mercaptoethanol and distilled water at a ratio of **5:5:2:88**.

The following example would prepare enough **Sample Extraction Solution** to assay 40 samples:

Sample Buffer (20X Concentrate) (F)	40 mL
Extraction Component A (20X Concentrate) (H)	40 mL
2-Mercaptoethanol	16 mL
Distilled water	704 mL
<hr/>	
Total	800 mL

Note:

- Extraction Component A (H)** may produce crystals after refrigerated storage. These crystals must be re-dissolved completely in a water bath at 30-37°C (86-99°F) prior to use.
The fully re-dissolved **Extraction Component A (H)** can be stored at 20-25°C (68-77°F).
- Sample Extraction Solution** can be stored at 4°C (39°F) and is used to prepare **Diluent II**.
If the **Sample Extraction Solution** forms a precipitate after refrigerated storage, then the solution must be warmed in a water bath at 20-25°C (68-77°F) to re-dissolve the precipitate prior to use.
- When testing food samples containing tannin and polyphenol (tea, cocoa, buckwheat, fruits, almond, lentil, sesame, etc.), 2% PVP is added to the **Sample Extraction Solution**.

Diluent I

Diluent I is used in the preparation of **Diluent II** and for diluting the **Sample Extract** in the LOW RANGE ASSAY PROTOCOL.

Dilute **Sample Buffer (F)** 20-fold with distilled water, for example:

Sample Buffer (20X Concentrate) (F)	5 mL
Distilled water	95 mL
<hr/>	
Total	100 mL

Diluent II

Diluent II is used to prepare the **Working Wheat Standard**, to further dilute the **Working Sample Solution** in the LOW RANGE ASSAY and to dilute the **Sample Extract** in the HIGH RANGE ASSAY PROTOCOL.

Dilute **Sample Extraction Solution** 20-fold with **Diluent I**, for example:

Sample Extraction Solution	2 mL
Diluent I	38 mL
Total	40 mL

Washing Solution

Dilute **Wash Buffer (G)** 20-fold with distilled water, and gently mix until the solution is homogeneous.

Wheat Protein Standard (50 ppb) (B)

The **Wheat Protein Standard** solution is used as a 50 ppb standard and to prepare the diluted **Working Wheat Standard** solution described for the LOW RANGE ASSAY PROTOCOL and HIGH RANGE ASSAY PROTOCOL.

The Wheat Protein Standard meets the reference material criteria of wheat required in Japan.

LOW RANGE ASSAY PROTOCOL

(0-20 ppm wheat protein, 0-17 ppm gluten)

The use of disposable polypropylene tubes is strongly recommended, and care should be taken to thoroughly clean all equipment and materials to eliminate cross-contamination, since the assay is highly sensitive.

a. Sample Preparation

Extraction of wheat protein from the test food sample can be performed in two different ways, depending on the user's convenience.

Short Time Extraction Method (to quantify wheat proteins in raw materials and finished products, including processed foods for allergen analysis where the results are required the same day).

1. Grind and mix the test food sample to homogeneity with a contamination-free homogenizer/blender.
2. Add 1.0 g or 1.0 mL of the homogenized sample to a disposable polypropylene centrifuge tube, containing 19 mL of **Sample Extraction Solution**, cap tightly and vortex for 30 seconds.
3. Incubate capped tube in a boiling water bath at 100°C (212°F) for 10 minutes.
4. Place the tube in running water to cool it down to ambient temperature (approximately 10 minutes).
5. Vortex the tube for 30 seconds.

Note: Check the fluid pH with pH test paper when using strongly acidic or alkaline samples and neutralize (pH 6-8) with HCl or NaOH, if required.

6. Centrifuge the tube at 3,000×g for 20 minutes at 20-25°C (68-77°F), and retain the supernatant as **Sample Extract**. (Filter the supernatant with filter paper, if necessary). Dilute the **Sample Extract** by 20-fold with **Diluent I** (see REAGENT PREPARATION), and use the extracted diluted solution as the **Working Sample Solution** in the ELISA.

Note: If further sample dilution is required, dilute the **Working Sample Solution** with **Diluent II**.

Overnight Extraction Method (to quantify wheat proteins in raw materials and finished products, including processed foods for allergen analysis where high sensitivity is required).

[This method has been validated as an official Japanese allergen analysis method. (3, 4)]

1. Grind and mix the test food sample to homogeneity with a contamination-free homogenizer/blender.
2. Add 1.0 g or 1 mL of the homogenized sample to a disposable polypropylene centrifuge tube, containing 19 mL of **Sample Extraction Solution**, cap tightly and vortex for 30 seconds.
3. Fix the capped centrifuge tube horizontally to a shaker, and oscillate at room temperature for at least 12 hours at 90-110 rpm, with a reciprocating motion of about 3 cm.

Note: Check the fluid pH with pH test paper when using strongly acidic or alkaline samples and neutralize (pH 6-8) with HCl or NaOH, if required.

4. Centrifuge the tube at 3,000×g for 20 minutes at 20-25°C (68-77°F), and retain the supernatant as **Sample Extract**. (Filter the supernatant with filter paper, if necessary).
5. Dilute the **Sample Extract** by 20-fold with **Diluent I** (see REAGENT PREPARATION), and use the extracted diluted solution as the **Working Sample Solution** in the ELISA.

Note: If further sample dilution is required, dilute the **Working Sample Solution** with **Diluent II**.

b. Working Wheat Standard Preparation

1. Dispense 0.5 mL **Diluent II** into six polypropylene micro tubes labeled 0.78, 1.56, 3.13, 6.25, 12.5 and 25 ppb.
2. Dispense 0.5 mL of 50 ng/mL **Wheat Protein Standard (B)** into the 25 ppb tube, and mix thoroughly.
3. Dispense 0.5 mL of the freshly-prepared 25 ppb standard into the 12.5 ppb tube, and mix thoroughly.
4. Repeat this 2-fold dilution series to prepare 6.25, 3.13, 1.56, and 0.78 ppb standards.
5. Dispense 0.5 mL of **Diluent II** into the polypropylene micro tube labeled 0 ppb.

c. ELISA

(First reaction)

1. Equilibrate the sealed **Antibody-coated Microplate Module (A)** to room temperature (20-25°C, 68-77°F) before it is unsealed. Fix the module to the mounting frame (supplied).
2. Dispense 100 µL of standards (0, 0.78, 1.56, 3.13, 6.25, 12.5, 25 and 50 ppb **Working Wheat Protein Standard**) and **Working Sample Solution**, in (at least) duplicate into each well.

Note: *Samples presumed to contain greater than 50 ppb of wheat protein should be diluted with **Diluent II**.*

3. Cover the microplate with a microplate cover (supplied).
4. Incubate for 1 hour at 20-25°C (68-77°F)

(Second reaction)

1. Completely remove the solution from each well by aspiration. (Or, pour the liquid out of the wells and vigorously tap the microplate frame upside down on paper towels to ensure complete removal of liquid from wells).
2. Wash six times, by the addition of 300 µL **Washing Solution** per well followed by aspiration. After washing, remove any remaining solution by inverting and tapping the plate on a clean paper towel. (Or, fill all the wells with 300 µL of the **Washing Solution** and pour out the liquid and tap. Repeat five more times).
3. Add 100 µL of **Enzyme-conjugated Antibody (C)** to each well.
4. Cover the microplate with a microplate cover and incubate for precisely 30 minutes at 20-25°C (68-77°F).

(Enzyme reaction)

1. Completely aspirate the well contents and wash six times by filling with 300 µL **Washing Solution** per well, and then aspirating. After the sixth wash, remove any remaining solution by inverting and tapping the plate on a clean paper towel. (Or, completely pour the liquid out of the wells, then fill all the wells with 300 µL of the **Washing Solution**, pour out the liquid again and tap. Repeat five more times).
2. Immediately dispense 100 µL of **Enzyme Substrate (D)** into each well.
3. Cover the microplate with the microplate cover and incubate for precisely 30 minutes at 20-25°C (68-77°F). During the enzyme reaction, avoid exposing the microplate to light.
4. Stop the enzyme reaction by adding 100 µL of **Stop Solution (E)** to each well.
5. Immediately measure absorbance using a microplate reader (measure at 450 nm, subtract a reference wavelength between 600 and 650 nm).

Note: The absorbance must be measured within 30 minutes after stopping the enzyme reaction.

Results and Interpretation

1. Determine the mean absorbance for each set of **Working Sample Solution** and **Working Wheat Protein Standard**. Use graphing software, the built-in graphing feature of the microplate reader, or graph paper to construct a standard curve by plotting the mean absorbance value for each standard on the Y axis versus the corresponding **Working Wheat Protein Standard** concentration on the X axis.
Note: A standard curve should be generated for each assay.
2. The wheat protein concentration in the **Working Sample Solution** is interpolated from the standard curve using the mean absorbance value of each observation.
3. If the mean absorbance of the **Working Sample Solution** is greater than the absorbance of the 50 ppb standard, increase the dilution of the **Working Sample Solution** to 40-fold or greater, if required, and assay again.

The wheat protein content in a sample, in ppm, can be estimated using the following formula:

$$\text{Wheat protein content (ppm)} = \text{OV} \times \text{Dilution A} \times \text{Dilution B} \times 1/1,000$$

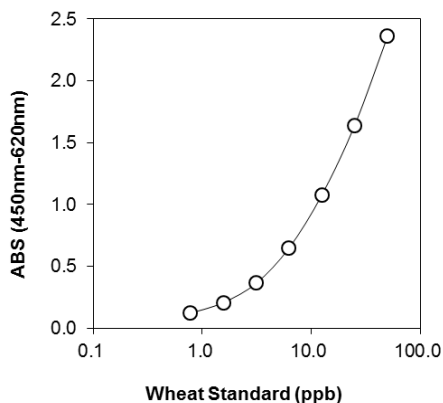
OV: Observed value (ppb)

Dilution A: Dilution for LOW RANGE ASSAY PROTOCOL a.2, normally 20-fold.

Dilution B: Dilution for LOW RANGE ASSAY PROTOCOL Short Time Extraction

Method a.7 or Overnight Extraction Method a.5, normally 20-fold.

Typical Standard Curve



Conversion to gluten

Gluten is widely considered to represent about 85% of wheat proteins (5-8). Therefore, the gluten content in wheat, barley, and rye of the test food sample can be estimated with a Wheat/Gluten ELISA kit using a conversion factor of 0.85 ng gluten per ng wheat protein and the following formula:

$$\text{Gluten content (ppm)} = \text{OV} \times \text{Dilution A} \times \text{Dilution B} \times 0.85 \times 1/1,000$$

OV: Observed value (ppb)

Dilution A: Dilution for LOW RANGE ASSAY PROTOCOL a. 2, normally 20-fold.

Dilution B: Dilution for LOW RANGE ASSAY PROTOCOL Short Time Extraction Method a.7 or Overnight Extraction Method a.5, normally 20-fold.

Summary of Procedure

(Extraction procedure)

Short Time Extraction Method

Grind/mince the samples

↓

Weigh 1.0 g or 1.0 mL sample in a tube, add 19 mL ***Sample Extraction Solution***.

↓

Incubate capped tube in boiling water for 10 minutes.

↓

Cool down the tube and vortex for 30 sec.

↓

Centrifuge, and filter the supernatant if necessary.

↓

Dilute ***Sample Extract*** 20-fold with ***Diluent I***.

Overnight Extraction Method

Grind/mince the samples

↓

Weigh 1.0 g or 1.0 mL sample in a tube, add 19 mL ***Sample Extraction Solution***.

↓

Fix the tube horizontally to a shaker, and oscillate at room temperature overnight.

↓

Centrifuge, and filter the supernatant if necessary.

↓

Dilute ***Sample Extract*** 20-fold with ***Diluent I***

(ELISA procedure)

Pipette 100 µL of **Working Wheat Protein Standards** and **Working Sample Solution** into individual wells in a microplate.

↓

Incubate the wrapped microplate for 1 hour at 20-25°C (68-77°F).

↓

Wash the wells 6 times with **Washing Solution**.

↓

Dispense 100 µL of **Enzyme-conjugated Antibody (C)** into each well.

↓

Incubate the microplate for 30 min at 20-25°C (68-77°F).

↓

Wash the wells 6 times with **Washing Solution**.

↓

Dispense 100 µL of **Enzyme Substrate (D)** into each well.

↓

Incubate the reaction for 30 min at 20-25°C (68-77°F) in the dark.

↓

Stop the enzyme reaction by adding 100 µL of **Stop Solution (E)** into each well.

↓

Measure absorbance at 450 nm.

↓

Calculate the concentration of wheat protein using the standard curve.

HIGH RANGE ASSAY PROTOCOL (0-68 ppm gluten)

To obtain accurate values, samples with a reading of 17 ppm gluten or higher should be diluted and an assay using the LOW RANGE ASSAY PROTOCOL should be carried out.

a. Sample Preparation

Extraction of wheat protein from the test food sample can be performed by two different methods depending on the user's convenience.

*Note: Please note procedural differences between the LOW RANGE ASSAY and HIGH RANGE ASSAY for the Dilution buffer and dilution factor of the **Sample Extract**.*

Short Time Extraction Method (to quantify the gluten content in raw materials and gluten free products, including processed foods and where the results are required the same day).

1. Grind and mix the test food sample to homogeneity with a contamination-free homogenizer/blender.
2. Add 1.0 g or 1.0 mL of the homogenized sample to a disposable polypropylene centrifuge tube, containing 19 mL of **Sample Extraction Solution**, cap tightly and vortex for 30 seconds.
3. Incubate capped tube in a boiling water bath at 100°C (212°F) for 10 minutes.
4. Place the tube in running water to cool it down to ambient temperature (approximately 10 minutes).
5. Vortex the tube for 30 seconds.

Note: Check the fluid pH with pH test paper when using strongly acidic or alkaline samples and neutralize (pH 6-8) with HCl or NaOH, if required.

6. Centrifuge the tube at 3,000xg for 20 minutes at 20-25°C (68-77°F), and retain the supernatant as **Sample Extract**. (Filter the supernatant with filter paper, if necessary).
7. Dilute the **Sample Extract** by 80-fold with **Diluent II** (see REAGENT PREPARATION), and the extracted diluted solution is used as the **Working Sample Solution** in the ELISA. Example of a **Working Sample Solution** preparation:

Sample Extract	0.025 mL
Diluent II	1.975 mL
<hr/>	
Total	2 mL

Overnight Extraction Method (to quantify the gluten content in raw materials and gluten free products, including processed foods).

1. Grind and mix the test food sample to homogeneity with a contamination-free homogenizer/blender.

2. Add 1.0 g or 1 mL of the homogenized sample to a disposable polypropylene centrifuge tube, containing 19 mL of **Sample Extraction Solution**, cap tightly and vortex for 30 seconds.
3. Fix the capped centrifuge tube horizontally to a shaker, and oscillate at room temperature for at least 12 hours at 90-110 rpm with a reciprocating motion of about 3 cm.

Note: Check the fluid pH with pH test paper when using strongly acidic or alkaline samples and neutralize (pH 6-8) with HCl or NaOH, if required.

4. Centrifuge the tube at 3,000×g for 20 minutes at 20-25°C (68-77°F), and retain the supernatant as **Sample Extract**. (Filter the supernatant with filter paper, if necessary).
 5. Dilute the **Sample Extract** by 80-fold with **Diluent II** (see REAGENT PREPARATION), and the extracted diluted solution is used as the **Working Sample Solution** in the ELISA.
- Example of a **Working Sample Solution** preparation:

Sample Extract	0.025 mL
Diluent II	1.975 mL
Total	2 mL

b. Working Wheat Protein Standard Preparation

1. Dispense 0.5 mL **Diluent II** into six polypropylene micro tubes labeled 0.78, 1.56, 3.13, 6.25, 12.5 and 25 ppb.
2. Dispense 0.5 mL of 50 ng/mL
3. **Wheat Protein Standard (B)** into the 25 ppb tube, and mix thoroughly.
4. Dispense 0.5 mL of the freshly-prepared 25 ppb standard into the 12.5 ppb tube, and mix thoroughly.
5. Repeat this 2-fold dilution series to prepare 6.25, 3.13, 1.56, and 0.78 ppb standards.
6. Dispense 0.5 mL of **Diluent II** into the polypropylene micro tube labeled 0 ppb.

c. ELISA

(First reaction)

1. Equilibrate the **Antibody-coated Microplate Module (A)** to room temperature (20-25°C, 68-77°F) before unsealing. Fix the module to the mounting frame (supplied).
2. Dispense 100 µL of the standards (0, 0.78, 1.56, 3.13, 6.25, 12.5, 25 and 50 ppb **Working Wheat Protein Standard**) and **Working Sample Solution**, into each well, in (at least) duplicate.

Note: Samples presumed to contain greater than 50 ppb of wheat protein should be diluted with **Diluent II**.

3. Cover the microplate with a microplate cover (supplied).

4. Incubate for 1 hour at 20-25°C (68-77°F).

(Second reaction)

1. Remove the solution completely from each well by aspiration. (Or, pour the liquid out of the wells and vigorously tap the microplate frame upside down on paper towels to ensure complete removal of liquid from wells).
2. Wash six times, by adding 300 µL **Washing Solution** per well followed by aspiration. After washing, remove any remaining solution by inverting and tapping the plate on a clean paper towel. (Or, fill all the wells with 300 µL of the **Washing Solution** and pour out the liquid again and tap. Repeat five more times).
3. Add 100 µL of **Enzyme-conjugated Antibody (C)** to each well.
4. Cover the microplate with a microplate cover and incubate for precisely 30 minutes at 20-25°C (68-77°F).

(Enzyme reaction)

1. Completely aspirate the well contents and wash six times by filling with 300 µL of **Washing Solution** per well, and then aspirating. After the sixth wash, remove any remaining solution by inverting and tapping the plate on a clean paper towel. (Or, completely pour the liquid out of the wells, then fill all the wells with 300 µL of the **Washing Solution**, pour out the liquid again and tap. Repeat five more times).
2. Immediately dispense 100 µL of **Enzyme Substrate (D)** into each well.
3. Cover the microplate with the microplate cover and incubate for precisely 30 minutes at 20-25°C (68-77°F). During the enzyme reaction, avoid exposing the microplate to light.
4. Stop the enzyme reaction by adding 100 µL of **Stop Solution (E)** to each well.
5. Immediately measure absorbance using a microplate reader (measure at 450 nm and subtract a reference wavelength between 600 and 650 nm).

Note: *The absorbance must be measured within 30 minutes after stopping the enzyme reaction.*

Results and Interpretation

1. Determine the mean absorbance for each set of **Working Sample Solution** and **Working Wheat Protein Standard**. Use graphing software, the built-in graphing feature of the microplate reader, or graph paper to construct a standard curve by plotting the mean absorbance value for each standard on the Y axis versus the corresponding **Working Wheat Protein Standard** concentration on the X axis.

Note: A standard curve should be generated for each assay.

2. The wheat protein concentration in the **Working Sample Solution** is interpolated from the standard curve using the mean absorbance value of each observation.

The gluten content in a sample, in ppm, can be estimated using the following formula:

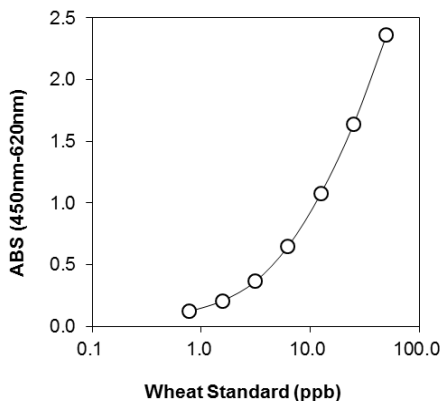
$$\text{Gluten content (ppm)} = \text{OV} \times \text{Dilution A} \times \text{Dilution B} \times 0.85 \times 1/1,000$$

OV: Observed value (ppb)

Dilution A: Dilution for HIGH RANGE ASSAY PROTOCOL a.2, normally 20-fold.

Dilution B: Dilution for HIGH RANGE ASSAY PROTOCOL Short Time Extraction Method a.7 or Overnight Extraction Method a.5, normally 80-fold.

Typical Standard Curve



Summary of Procedure

(Extraction procedure)

Short Time Extraction Method

Grind/mince the samples

↓

Weigh 1.0 g or 1.0 mL sample in a tube, add 19 mL **Sample Extraction Solution**.

↓

Incubate capped tube in boiling water for 10 minutes.

↓

Cool down the tube and vortex for 30 sec.

↓

Centrifuge, and filter the supernatant if necessary.

↓

Dilute **Sample Extract** 80-fold with **Diluent II**.

Overnight Extraction Method

Grind/mince the samples

↓

Weigh 1.0 g or 1.0 mL sample in a tube, add 19 mL **Sample Extraction Solution**.

↓

Fix the tube horizontally to a shaker, and oscillate at room temperature overnight.

↓

Centrifuge, and filter the supernatant if necessary.

↓

Dilute **Sample Extract** 80-fold with **Diluent II**.

(ELISA procedure)

Pipette 100 µL of **Working Wheat Protein Standard** and **Working Sample Solution** into individual wells of the microplate.

↓

Incubate the wrapped microplate for 1 hour at 20-25°C (68-77°F).

↓

Wash the wells 6 times with **Washing Solution**.

↓

Dispense 100 µL of **Enzyme-conjugated Antibody (C)**.

↓

Incubate the microplate for 30 min at 20-25°C (68-77°F).

↓

Wash the wells 6 times with **Washing Solution**.

↓

Dispense 100 µL of **Enzyme Substrate (D)**.

↓

Incubate reaction for 30 min at 20-25°C (68-77°F) in the dark.

↓

Stop the enzyme reaction by adding 100 µL of **Stop Solution (E)**.

↓

Measure absorbance at 450 nm.

↓

Calculate the concentration of wheat protein using the standard curve.

STORAGE CONDITIONS AND EXPIRY OF KIT

1. Store the kit at 2-8°C (35-46°F), DO NOT FREEZE!
2. Use the kit at 20-25°C (68-77°F), while minimizing exposure time at this temperature.
The kit must be returned to storage at 2-8°C (35-46°F) as soon as possible. Do not expose this kit to temperatures in excess of 25°C (77°F)!
3. The shelf life is one year from production.
4. Do not use the kit after the expiration date indicated on the outside box.

WARNINGS AND PRECAUTIONS

(General)

1. Wheat protein is used in this kit. Users who are allergic to wheat protein should handle kit reagents and perform the assay with great care. In case of an allergic

reaction, such as sneezing or itching, seek medical attention if the reaction is serious or prolonged.

2. This product is recommended for use only by personnel trained in analytical sample handling techniques, and is meant to be used in accordance with good laboratory practices.
3. Direct contact with **Stop Solution (E)** should be strictly avoided. In case of contact, immediately flush affected area with copious amounts of water, and seek medical attention if necessary. In case of ingestion, call the poison control center and obtain first aid treatment.
4. Because all chemicals should be considered potentially hazardous, it is advisable to wear suitable protective clothing, goggles and gloves.
5. This product uses 2-mercaptoethanol which is categorized as a hazardous substance (CAS No.60-24-2). Perform the assay under a well-ventilated hood, and handle the reagents with caution.
6. Because the assay is highly sensitive, all the procedures should be performed in a clean environment using uncontaminated equipment/devices and tubes/containers in order to minimize the risk of cross-contamination from previous analyses.
7. It is desirable to implement an internal quality control (QC) Morinaga can provide a QC sample for this ELISA to confirm the validity of the result.

(ELISA)

1. Do not combine reagents from different lots or other kits.
2. All reagents should be equilibrated at 20-25°C (68-77°F) before use.
3. A standard curve should be generated for each assay concurrently with the samples.
4. Assays should be performed at least in duplicate to ensure confidence in the measured values.
5. Care should be taken to pipette standard solutions and samples accurately.
6. Follow all procedures carefully.
7. Washing must be thorough in order to minimize background readings. Complete removal of reagents from the microplate wells is essential.
8. The enzyme substrate reaction should be performed shielded from light.

Note: *In the case of processed foods, the detection efficiency or sensitivity of the assay may decrease, due to denaturation and insolubilization of wheat proteins. Therefore, food samples that give a negative result may still contain wheat material which is either unreactive or present at concentrations below the limit of detection. It should not be*

assumed that such foods are wheat/gluten free

Reagent preparation

	Reagent	Preparation procedure
A	Antibody-coated Microplate Module	Equilibrate to 20-25°C (68-77°F) before opening. Use immediately after opening.
B	Wheat Protein Standard (50 ppb)	Equilibrate to 20-25°C (68-77°F).
	Working Wheat Standards (0.78, 1.56, 3.13, 6.25, 12.5 and 25 ppb)	Sequentially dilute Wheat Protein Standard (50 ppb) with Diluent II .
C	Enzyme-conjugated Antibody	Equilibrate to 20-25°C (68-77°F).
D	Enzyme Substrate	Equilibrate to 20-25°C (68-77°F). Avoid exposure to light.
E	Stop Solution	Equilibrate to 20-25°C (68-77°F).
H	Extraction Component A	Equilibrate to 20-25°C (68-77°F), if necessary dissolve any remaining crystals in a water bath at 30-37°C (86-99°F).
	Sample Extraction Solution	Mix Sample Buffer , Extraction Component A , 2-mercaptoethanol and distilled water at a ratio of 5:5:2:88.
	Diluent I	Dilute Sample Buffer 20-fold with distilled water.
	Diluent II	Dilute Sample Extraction Solution 20-fold with Diluent I
	Washing Solution	Dilute Wash Buffer 20-fold with distilled water.

REFERENCES

1. Y. Watanabe, K Aburatani, T Mizumura, M Sakai, S Muraoka, S Mamegoshi and T Honjoh (2005), "Novel ELISA for the detection of raw and processed egg using extraction buffer containing a surfactant and a reducing agent", *Journal of Immunological Methods*, Vol. 300, pp. 115-123
2. Federal Register Vol.78, No.150, P47154
3. R. Matsuda *et al.*(2006), "Interlaboratory evaluation of two enzyme-linked immunosorbent assay kits for the detection of egg, milk, wheat, buckwheat, and peanut in foods.", *Journal of AOAC International*, Vol. 89, pp.1600-1608
4. H.Doi *et al.*(2010), "Inter-laboratory evaluation for improved ELISA method of wheat protein.", *Japanese Journal of Food Chemistry and Safety*, Vol.17, pp.12-17
5. T. B. Osborne (1924), *The Vegetable Proteins*, Longmans green and Co., London, UK
6. John Holme (1966), "A review of wheat flour proteins and their functional properties", *The Bakers Digest*, Vol.40, pp.38-42
7. S. Shibata, and T. Nakae (Ed.) (1990), *Komugikoseihinno chisiki [Knowledge of wheat products]*, Japan, Saiwai Shobo
8. Nihon mugirui kenkyukai (Ed.) (1964), *Komugiko-sono genryo to kakohin [Wheat-material and processing]*, Japan, Yuni Ato

TECHNICAL ASSISTANCE

For further technical assistance or troubleshooting advice, contact Morinaga Institute of Biological Science, Inc. or your local distributor.

Morinaga Institute of Biological Science, Inc.

Telephone: +81-45-791-7674

Fax: +81-45-791-7675

E-mail: info_miobs_e@morinaga.co.jp

Website: <https://www.miobs-e.com>

WARRANTY

Morinaga Institute of Biological Science, Inc. makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. Buyer assumes all risk and liability resulting from the use of this product. There is no warranty of merchantability of the product, or of the fitness of the product for any purpose. Morinaga Institute of Biological Science, Inc. agrees to replace any defective product, but expressly disclaims liability for damages, including special or consequential damages, or expenses arising directly or indirectly from the use of this product.