V.9/Jan. 2026

Egg (Ovalbumin) ELISA Kit Ⅱ (Cat# M2111)
Total Milk ELISA Kit Ⅱ (Cat# M2122)
Beta-lactoglobulin ELISA Kit Ⅱ (Cat# M2112)
Casein ELISA Kit Ⅱ (Cat# M2113)
Wheat/Gluten(Gliadin) ELISA Kit Ⅱ (Cat# M2114)
Buckwheat ELISA Kit Ⅱ (Cat# M2115)
Peanut ELISA Kit Ⅱ (Cat# M2116)
Walnut ELISA Kit Ⅱ (Cat# M2124)
Cashew ELISA Kit Ⅱ (Cat# M2125)
Soya ELISA Kit Ⅱ (Cat# M2117)

The Quantitative Determination for
Protein of Allergic Ingredients in Food

96 Assays

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

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TABLE OF CONTENTS

INTENDED USE AND USER 19

PRINCIPLE OF ELISA 19

KIT COMPONENTS 20

REQUIRED MATERIALS (NOT INCLUDED) 20

PERFORMANCE CHARACTERISTICS 20

REAGENT PREPARATION 21

ASSAY PROTOCOL 22

Summary of Procedure 24

RESULTS AND INTERPRETATION 25

CONVERSION FACTOR FOR PEANUT STANDARD TO NIST PEANUT BUTTER SRM2387... 25

TYPICAL STANDARD CURVE 26

STORAGE CONDITIONS AND EXPIRY OF KIT 27

WARNINGS AND PRECAUTIONS 27

TECHNICAL ASSISTANCE 28

WARRANTY 28

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

These ELISA kits are sandwich enzyme immunoassays for the quantitative determination of individual proteins of allergic ingredients in processed or unprocessed food. These kits are designed to be used by quality control personnel or other trained professionals.

Table 1 : Analytes and Test Results for each kit

Kit	Analyte	Test Result
Egg(Ovalbumin) ELISA Kit Ⅱ	Ovalbumin	Whole egg protein
Total Milk ELISA Kit Ⅱ	Casein and Beta-lactoglobulin	Whole milk protein
Beta-lactoglobulin ELISA Kit Ⅱ	Beta-lactoglobulin	Whole milk protein
Casein ELISA Kit Ⅱ	Casein	Whole milk protein
Wheat/Gluten(Gliadin) ELISA Kit Ⅱ	Gliadin	Whole wheat protein
Buckwheat ELISA Kit Ⅱ	Partially purified buckwheat protein	Whole buckwheat protein
Peanut ELISA Kit Ⅱ	Partially purified peanut protein	Whole peanut protein
Walnut ELISA Kit Ⅱ	2S albumin	Whole walnut protein
Cashew ELISA Kit Ⅱ	2S albumin	Whole cashew protein
Soya ELISA Kit Ⅱ	Beta-conglycinin	Whole soya protein

PRINCIPLE OF ELISA

Proteins in the samples are extracted, centrifuged and filtered prior to the sandwich ELISA. Analyte is bound to the antibody coated wells of the microplate module. This results in the formation of an antigen-antibody complex in the wells. Unbound materials are removed by washing. Subsequently, the enzyme-conjugated antibody is bound to the already bound antigen-antibody complex, forming an antibody-antigen-antibody sandwich. A second washing step removes the excess conjugated antibody. Addition of enzyme substrate results in color development due to the enzyme bound to the complex. After addition of the stop solution, the color intensity of the solutions can be determined by the absorbance at 450 nm. The intensity of the color developed is directly proportional to the concentration of protein of allergic ingredients in the food. The concentration of its protein corresponding to the measured absorbance is determined by preparing a standard curve, and adjusting for a further dilution factor if necessary.

KIT COMPONENTS

Label	Name of component	Content	Quantity
A	Reagent A (10X Concentrate)	100 mL	1 bottle
B	Reagent B (10X Concentrate)	100 mL	1 bottle
C	Reagent C (10X Concentrate)	100 mL	1 bottle
D	Antibody-coated Microplate Module	6X8 well modules	2 packs
E	Standard (50 ng/mL extracted protein solution)	1 mL	2 vials
F	Enzyme-conjugated Antibody	13 mL	1 bottle
G	Enzyme Substrate (TMB Solution)	13 mL	1 bottle
H	Stop Solution (1N Sulfuric Acid)	13 mL	1 bottle
I	Wash Buffer (20X Concentrate)	50 mL	1 bottle
	Frame for mounting the microplate module		1 piece
	Microplate cover		1 piece

REQUIRED MATERIALS (NOT INCLUDED)

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

PERFORMANCE CHARACTERISTICS

These ELISA kits are tools to determine the presence of each protein of allergic ingredients in foods such as canned juice, strawberry jam, retorted braised tomatoes and retorted baby food.

Sample preparation time	Approx. 10 min
Sample extraction time	Approx. 30 min, or overnight
Time for ELISA analysis	Approx. 2 h
Assay sensitivity	0.31 ppm (0.31 μg protein*/g food)
Assay range	0.78 to 50 ppb protein*
Intra-assay precision	< 10 %
Inter-assay precision	< 10 %

* Refer to the Test Result in Table 1 (p.19).

REAGENT PREPARATION

Sample Extraction Solution

Prepare the ***Sample Extraction Solution*** by mixing ***Reagent A (A)***, ***Reagent B (B)***, ***Reagent C (C)*** and distilled water at a ratio of 1:1:1:7.

The following example would prepare enough ***Sample Extraction Solution*** to assay 16 samples:

Reagent A (10X Concentrate) (A)	40 mL
Reagent B (10X Concentrate) (B)	40 mL
Reagent C (10X Concentrate) (C)	40 mL
Distilled water	280 mL
Total	400 mL

Note*:*

a. ***Reagent A (A)*** *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 Reagent A can be stored at 20-30 °C (68-86 °F).*

b. ***Sample Extraction Solution*** *can be stored at 4 °C for preparing* ***Diluent II****. If* ***Sample Extraction Solution*** *forms a precipitate after refrigerated storage, then the solution must be warmed in a water bath at 20-30 °C (68-86 °F) to re-dissolve the precipitate prior to use.*

Diluent I

Diluent I is used for preparing ***Diluent II*** and for diluting the ***Sample Extract.***

Dilute ***Reagent C (C)*** 10-fold with distilled water.

The following is an example of preparing ***Diluent I***:

Reagent C (10X Concentrate) (C)	5 mL
Distilled water	45 mL
Total	50 mL

Diluent II

Diluent II is used to prepare ***Working Standard***, and to further dilute the ***Working Sample Solution***.

Dilute ***Sample Extraction Solution*** 20-fold with ***Diluent I***.

The following is an example of preparing ***Diluent II***:

Sample Extraction Solution	1 mL
Diluent I	19 mL
Total	20 mL

Washing Solution

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

Standard (50 ng/mL) (E)

Standard (50 ng/mL) (E) is used as a 50 ppb standard as well as for preparing the diluted working standards described in ASSAY PROTOCOL b.

ASSAY PROTOCOL

It is strongly recommended to use disposable polypropylene tubes, and care should be taken to clean up all equipments and materials so as to be free from cross-contamination, since the assay is highly sensitive.

a. Sample Preparation

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

(Short Time Extraction Method)

1. Grind and mix up the test food sample to homogeneity with a contamination-free homogenizer/blender.
2. A disposable polypropylene centrifuge tube, containing 1.0 g of the homogenized sample and 19 mL of ***Sample Extraction Solution,*** is capped tightly and vortexed 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.
6. Check the fluid pH with pH test paper, and neutralize (pH 6-8) with HCl or NaOH, if required.
7. Centrifuge the tube at 3,000×*g* for 20 minutes at 20-30 °C (68-86 °F), and the supernatant is retained as ***Sample Extract.*** (Filter the supernatant with filter paper, if necessary.)
8. Dilute the ***Sample Extract*** 20-fold with ***Diluent I*** (see REAGENT PREPARATION), and the diluted solution is used as the ***Working Sample Solution*** for ELISA.
   Note*: If further sample dilution is required, dilute the* ***Working Sample Solution*** *with* ***Diluent II***.

(Overnight Extraction Method)

1. Grind and mix up the test food sample to homogeneity with a contamination-free homogenizer/blender.
2. A disposable polypropylene centrifuge tube, containing 1.0 g of the homogenized sample and 19 mL of ***Sample Extraction Solution***, is capped tightly and vortexed for 30 seconds.
3. Fix the capped centrifuge tube to a shaker horizontally, and oscillate at room temperature overnight (for at least 12 hours at 90-110 rpm) with a reciprocating motion of about 3 cm.
4. Follow the procedures 6-8 for ***Short Time Extraction Method***.

b. Working Standard Preparation

1. Dispense 0.5 mL of ***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 ***Standard (50 ng/mL) (E)*** 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. Unseal the ***Antibody-coated Microplate Module (D)*** after equilibrated to room temperature (20-30 °C, 68-86 °F). Fix the module to the mounting frame (supplied).
2. Into each well, dispense 100 μL of standards (0, 0.78, 1.56, 3.13, 6.25, 12.5, 25 and 50 ppb ***Working Standard***) and ***Working Sample Solution***, in (at least) duplicate.
   Note*: Working Sample Solution presumed to be greater than 50 ppb should be diluted with* **Diluent II** *(ASSAY PROTOCOL a.8, Note)*.
3. Cover the microplate with a microplate cover (supplied).
4. Incubate for 1 hour at 20-30 °C (68-86 °F).

(Second reaction)

1. Remove the solution completely from each well by aspiration. (OR: Carefully pour the liquid out of the wells and tap the microplate frame upside down vigorously five times on paper towels to ensure complete removal of liquid from wells.)
2. Wash six times, by adding 300 μL of ***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 (300 μL of the ***Washing Solution*** in each), pour out the liquid again and tap. Repeat five more times.)
   Note: When using an automated plate washer, increase the volume of ***Washing Solution*** per well to a maximum of 400 µL to ensure thorough washing.
3. Add 100 μL of ***Enzyme-conjugated Antibody (F)*** to each well.
4. Cover the microplate with a microplate cover and incubate for precisely 30 minutes at 20-30 °C (68-86 °F).

(Enzyme reaction)

1. Completely aspirate the well contents and wash six times by filling with 300 μL of ***Washing Solution*** per well, and aspirate. After the sixth washing, 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 (G)*** into each well.
3. Cover the microplate with the microplate cover and incubate for precisely 20 minutes at 20-30 °C (68-86 °F). During the enzyme reaction, avoid exposing the microplate to light.
4. Stop the enzyme reaction by adding 100 μL of ***Stop Solution (H)*** to each well.
5. Immediately measure absorbance using a microplate reader at 450 nm, subtract a reference wavelength between 600 and 650 nm, inclusive.
   Note*: The absorbance must be measured within 30 minutes after stopping the enzyme reaction.*

Summary of Procedure

(Extraction procedure)

Short Time Extraction Method

Grind/mince the samples

↓

Weigh 1.0 g 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.

↓

Adjust pH to 6.0-8.0.

↓

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 sample in a tube, add 19 mL ***Sample Extraction Solution***.

↓

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

↓

Adjust pH to 6.0-8.0.

↓

Centrifuge and filter the supernatant if necessary.

↓

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

(ELISA procedure)

Pipette 100 μL ***Working Standard*** and ***Working Sample Solution***.

↓

Incubate the microplate for 1 hour at 20-30 °C (68-86 °F).

↓

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

↓

Dispense 100 μL ***Enzyme-conjugated Antibody (F)***.

↓

Incubate the microplate for 30 min at 20-30 °C (68-86 °F).

↓

Wash the wells 6 times.

↓

Dispense 100 μL ***Enzyme Substrate (G)***.

↓

Incubate reaction for 20 min at 20-30 °C (68-86 °F) in the dark.

↓

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

↓

Measure absorbance at 450 nm, subtract a reference wavelength between 600 and 650 nm, inclusive.

↓

Calculate the results using the standard curve.

RESULTS AND INTERPRETATION

1. Determine the mean absorbance for each set of ***Working Sample Solution*** and ***Working Standard***. Plot and calculate the test results using graphing software. A standard curve should be generated for each assay. For optimal data processing, a 4-parameter curve fit is recommended. Alternatively, a Cubic Spline curve fit is also available.
2. The protein concentration in the ***Working Sample Solution*** is interpolated from the standard curve using the mean absorbance of each observation.
3. If the mean absorbance of ***Working Sample Solution*** is greater than the absorbance of the 50 ppb standard, increase the dilution of ***Working Sample Solution*** to 40-fold or greater as appropriate instead of 20-fold, and then assay again.

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

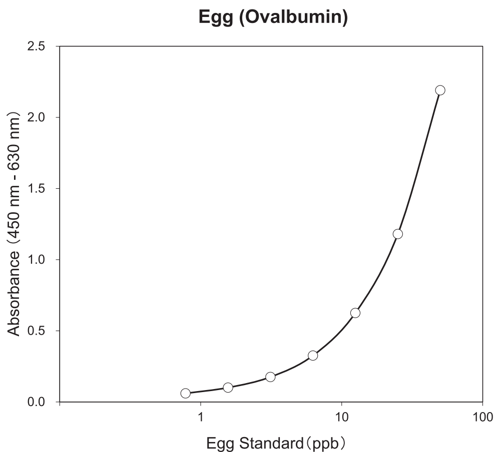
Protein content (ppm) = OV × Dilution A × Dilution B × 1/1,000

OV: Observed value (ppb)
Dilution A: Dilution for ASSAY PROTOCOL a.2, nominal 20-fold.
Dilution B: Dilution for ASSAY PROTOCOL a.8, nominal 20-fold.

CONVERSION FACTOR FOR PEANUT STANDARD TO NIST PEANUT BUTTER SRM2387

Please find the conversion factor for peanut standard to NIST peanut butter SRM2387 with the lot number in web site as follows:
https://www.morinaga-biosci.com
Home > Products > Food Allergen ELISA Kit Ⅱ> Peanut ELISA Kit Ⅱ Cat. M2116

TYPICAL STANDARD CURVE



Note: Standard curves for other items will have a similar shape.

STORAGE CONDITIONS AND EXPIRY OF KIT

1. Store the kit at 2-8 °C (35-46 °F), but DO NOT FREEZE!
2. Use the kit at 20-30 °C (68-86 °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 30 °C (86 °F)!
3. Do not use the kit after the expiration date indicated on the outside box.

WARNINGS AND PRECAUTIONS

(General)

1. Food allergen are used in this kit. Users who are allergic to these proteins 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 (H)*** 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. 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.

(ELISA)

1. Do not combine reagents from different lots or other kits.
2. All reagents should be equilibrated at 20-30 °C (68-86 °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.
9. Do not touch the bottom of the plate. Cloudy bottom may prevent accurate absorbance measurements. If the bottom becomes cloudy due to fingerprints or other marks, wipe it thoroughly with a wiping paper before measuring absorbance.

***Note**: In the case of processed foods, the detection efficiency or sensitivity of the assay may decrease, due to denaturation and insolubilization of proteins. Therefore, food samples that give a negative result may still contain allergic ingredient which is either unreactive or present at concentrations below the limit of detection. It should not be assumed that such foods are <u>allergic ingredient free</u>.*

TECHNICAL ASSISTANCE

For further technical assistance or troubleshooting advice, contact Morinaga BioScience, Inc. or your local distributor.

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WARRANTY

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