V.7/Aug/2016

MORINAGA

Peanut ELISA Kit

For the quantitative determination of
peanut protein in food

Catalog #M2104
96 Assays

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

E-mail: info@miobs.com
Website: http://www.miobs-e.com/index.html

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TABLE OF CONTENTS

INTENDED USE AND USER..... 3

PRINCIPLE OF ELISA 3

KIT COMPONENTS 3

REQUIRED MATERIALS (NOT INCLUDED) 4

PERFORMANCE CHARACTERISTICS 4

REAGENT PREPARATION 5

BASE PROTOCOL (Official Japan Method)..... 6

Summary of Procedure 8

Result and Interpretation..... 9

ALTERNATE PROTOCOL (NIST SRM2387 peanut butter calibrated) 10

Summary of Procedure 12

Result and Interpretation..... 13

STORAGE CONDITIONS AND EXPIRY OF KIT 14

WARNINGS AND PRECAUTIONS 14

Reagent preparation 15

TECHNICAL ASSISTANCE 16

WARRANTY..... 16

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

Peanut ELISA Kit is a sandwich enzyme immunoassay for the quantitative determination of peanut protein in processed or unprocessed food. This kit is designed to be used by quality control personnel or other trained professionals.

PRINCIPLE OF ELISA

Peanut protein in the samples is extracted, centrifuged and filtered prior to the sandwich ELISA. The peanut protein (antigen) is bound to the anti-peanut protein polyclonal 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 anti-peanut protein antibody is bound to the already bound peanut protein-antibody complex, forming an antibody-antigen-antibody sandwich. The 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 the peanut protein in the food. The concentration of peanut 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	Antibody-coated Microplate Module	6X8 well modules	2 packs
B	Peanut Standard	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 50 to 1000 µL
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 sample
7. Tube racks
8. pH test paper
9. Clear-plastic wrap
10. Homogenizer/blender for sample preparation (if necessary)
11. Water bath for boiling or horizontal shaker, for sample extraction
12. Centrifuge
13. Vortex mixer
14. Aspirator for washing procedure, or optional microplate washer
15. Microplate reader with a 450 nm filter, and a filter for any wavelength from 600 to 650 nm, inclusive

PERFORMANCE CHARACTERISTICS

Peanut ELISA kit is a tool to determine the presence of peanut protein in foods such as breakfast cereal, cookie, ice cream and milk chocolate.

The kit protocol, range, and sensitivity will vary based on the method of calibration used for the standard. The Base Protocol is based on calibrating the standard to accepted Japanese reference material using defatted raw peanut. The standard value when calibrated using this method is 50 ng/mL.

The Alternative Protocol is based on calibrating the standard to NIST SRM2387 peanut butter, a commonly used reference material outside of Japan. The standard value when calibrated using this method is 160 ng/mL.

Sample preparation time	Approx. 10 min	
Sample extraction time	Approx. 30 min, or overnight	
Time for ELISA analysis	Approx. 2.5 h	
Assay sensitivity	Base Protocol	0.3 µg peanut protein/g food
	Alternate Protocol	0.5 µg peanut protein/g food
Detectable concentration range	Base Protocol	0.78 - 50 ng/mL peanut protein
	Alternate Protocol	1.25 - 80 ng/mL peanut protein
Intra-assay precision	< 10 %	
Inter-assay precision	< 10 %	
Recovery	100 +/- 25 %	

Specificity data are available. In need, please contact to Morinaga Institute of Biological Science, Inc. or your local distributor. Specificity results are the data obtained by commercially available materials, and vary somehow from lot to lot.

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
Total	800 mL

Note:
a. ***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*** *can be stored at 20-25°C (68-77°F).*
b. ***Sample Extraction Solution*** *can be stored at 4°C (39°F) 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 20-25°C (68-77°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 ***Sample Buffer (F)*** 20-fold with distilled water.
The following is an example of preparing ***Diluent I***:

Sample Buffer (20X Concentrate) (F)	5 mL
Distilled water	95 mL
Total	100 mL

Diluent II
Diluent II is used to prepare ***Working Peanut 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	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.

Peanut Standard (B)
Each kit comes with a ready to use liquid peanut standard. The value of the Peanut Standard is based on the calibration method used as described in the Performance Characteristics section.

BASE PROTOCOL (JAPANESE OFFICIAL METHOD)

The Base Protocol is based on calibrating the standard to accepted Japanese reference material using defatted raw peanut. The standard value when calibrated using this method is 50 ng/mL.

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 peanut protein 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. Expose the capped tube to 100°C (212°F) in boiling water for 10 minutes.
4. Place the tube in running water to cool it down to ambient temperature (it takes some 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 20-25°C (68-77°F), and the supernatant is retained as ***Sample Extract***. (Filter the supernatant with filter paper, if necessary.)
8. Dilute the ***Sample Extract*** by 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 Peanut 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 ng/mL.
2. Dispense 0.5 mL of 50 ng/mL ***Peanut Standard (B)*** into the 25 ng/mL tube, and mix thoroughly.
3. Dispense 0.5 mL of the freshly-prepared 25 ng/mL standard into the 12.5 ng/mL tube, and mix thoroughly.

4. Repeat this 2-fold dilution series to prepare 6.25, 3.13, 1.56, and 0.78 ng/mL standards.
5. Dispense 0.5 mL of ***Diluent II*** into the polypropylene micro tube labeled 0 ng/mL.

c. ELISA

(First reaction)

1. Unseal the ***Antibody-coated Microplate Module (A)*** after equilibrated to room temperature (20-25°C, 68-77°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 ng/mL ***Working Peanut Standard***) and ***Working Sample Solution***, in (at least) duplicate.
   Note*: Samples presumed to contain greater than 50 ng/mL of peanut protein should be diluted with* ***Diluent II*** *(ASSAY PROTOCOL a.8, Note).*
3. Cover the microplate with a microplate cover (supplied), and wrap them all by clear-plastic wrap to minimize the odor during incubation.
4. Incubate for 1 hour at 20-25℃ (68-77°F).

(Second reaction)

1. Carefully unwrap the plate, and then remove the solution completely from each well by aspiration. (OR: Carefully unwrap the plate, and pour the liquid out of the wells and tap the microplate frame upside down vigorously for five times 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 ***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 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 ***Enzyme Substrate (D)***.
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)***.
5. Immediately measure absorbance using a microplate reader (measure 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 – Base Protocol

(Extraction procedure)

Grind/mince the samples to homogeneity

Weigh 1.0 g ground (or liquid) sample in a clean centrifuge tube, add 19 mL ***Sample Extraction Solution***, cap the tube tightly, and vortex.

Short Time Extraction Method

-- Expose the capped tube to 100°C (212°F) in boiling water for 10 minutes.
-- Cool down the centrifuge tube in running water, and vortex for 30 sec.
-- Adjust pH to 6.0-8.0.

Overnight Extraction Method

-- 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.
-- Adjust pH to 6.0-8.0.

Centrifuge at 3,000 × *g* for 20 min at 20-25°C (68-77°F) and filter the supernatant if necessary.

↓

Dilute ***Sample Extract*** 20-fold with ***Diluent I***
(If necessary, dilute further using ***Diluent II***)

(ELISA procedure)

Attach the ***Antibody-coated Microplate Module (A)*** to the mounting frame

Pipette 100 µL ***Working Peanut Standard*** and ***Working Sample Solution*** into each well

↓

-- 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 ***Enzyme-conjugated Antibody (C)*** per 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 ***Enzyme Substrate (D)*** per well

↓

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

Stop the enzyme reaction by adding 100 µL ***Stop Solution (E)*** per well

↓

Measure absorbance at 450 nm
(Subtract the reading at a reference wavelength: any wavelength from 600 to 650 nm, inclusive)

↓

Calculate the concentration of peanut protein using the standard curve

* The wells are emptied completely by inverting and tapping the microplate on a clean paper towel.

Results and Interpretation

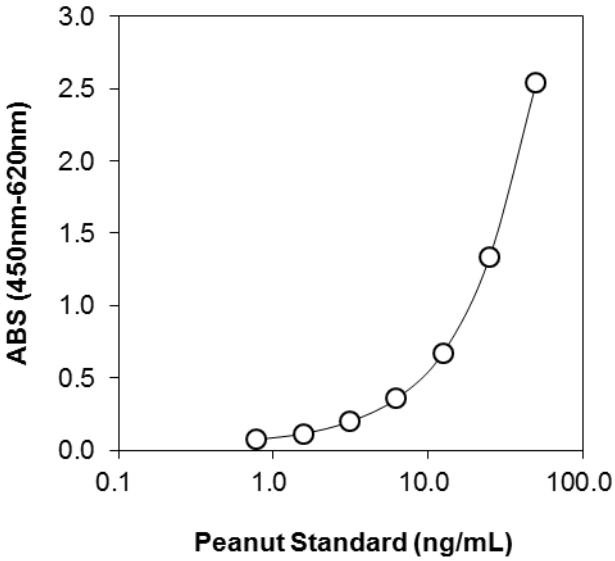
1. Determine the mean absorbance for each set of ***Working Sample Solution*** and ***Working Peanut Standard***. Use graphing software, the built-in graphing feature of the microplate reader, or graph paper to construct a standard curve by plotting the mean absorbance value for each standard on the Y axis versus the corresponding ***Working Peanut Standard*** concentration on the X axis.
   Note*: A standard curve should be generated for each assay.*
2. The peanut 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 ***Working Sample Solution*** is greater than the absorbance of the 50 ng/mL standard, increase the dilution of ***Working Sample Solution*** to 40-fold or greater as appropriate instead of 20-fold, and then assay again.

The peanut protein content in a sample, in µg/g, can be estimated using the following formula:

Peanut protein content (µg/g) = OV× Dilution A× Dilution B× 1/1,000

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

Typical Standard Curve



ALTERNATE PROTOCOL （NIST SRM2387 PEANUT BUTTER CALIBRATED)

The Alternative Protocol is based on calibrating the standard to NIST SRM2387 peanut butter, a commonly used reference material outside of Japan. The standard value when calibrated using this method is 160 ng/mL.

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 peanut protein 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. Expose the capped tube to 100°C (212°F) in boiling water for 10 minutes.
4. Place the tube in running water to cool it down to ambient temperature (it takes some 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 20-25°C (68-77°F), and the supernatant is retained as ***Sample Extract***. (Filter the supernatant with filter paper, if necessary.)
8. Dilute the ***Sample Extract*** by 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 Peanut Standard Preparation

1. Dispense 0.5 mL ***Diluent II*** into seven polypropylene micro tubes labeled 1.25, 2.5, 5, 10, 20, 40 and 80 ng/mL.
2. Dispense 0.5 mL of 160 ng/mL (NIST SRM2387 peanut butter calibrated value) ***Peanut Standard (B)*** into the 80 ng/mL tube, and mix thoroughly.

3. Dispense 0.5 mL of the freshly-prepared 80 ng/mL standard into the 40 ng/mL tube, and mix thoroughly.
4. Repeat this 2-fold dilution series to prepare 20, 10, 5, 2.5 and 1.25 ng/mL standards.
5. Dispense 0.5 mL of ***Diluent II*** into micro tube labeled 0 ng/mL.

c. ELISA

(First reaction)

1. Unseal the ***Antibody-coated Microplate Module (A)*** after equilibrated to room temperature (20-25°C, 68-77°F). Fix the module to the mounting frame (supplied).
2. Into each well, dispense 100 µL of standards (0, 1.25, 2.5, 5, 10, 20, 40 and 80 ng/mL ***Working Peanut Standard***) and ***Working Sample Solution***, in (at least) duplicate.
   Note*: Samples presumed to contain greater than 80 ng/mL of peanut protein should be diluted with* ***Diluent II*** *(ASSAY PROTOCOL a.8, Note).*
3. Cover the microplate with a microplate cover (supplied), and wrap them all by clear-plastic wrap to minimize the odor during incubation.
4. Incubate for 1 hour at 20-25℃ (68-77°F).

(Second reaction)

1. Carefully unwrap the plate, and then remove the solution completely from each well by aspiration. (OR: Carefully unwrap the plate, and pour the liquid out of the wells and tap the microplate frame upside down vigorously for five times 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 ***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 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 ***Enzyme Substrate (D)***.
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)***.
5. Immediately measure absorbance using a microplate reader (measure 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 – Alternate Protocol

(Extraction procedure)

Grind/mince the samples to homogeneity

Weigh 1.0 g ground (or liquid) sample in a clean centrifuge tube, add 19 mL ***Sample Extraction Solution***, cap the tube tightly, and vortex.

Short Time Extraction Method

-- Expose the capped tube to 100°C (212°F) in boiling water for 10 minutes.

-- Cool down the centrifuge tube in running water, and vortex for 30 sec.

-- Adjust pH to 6.0-8.0.

Overnight Extraction Method

-- 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.

-- Adjust pH to 6.0-8.0.

↓

Centrifuge at 3,000 × *g* for 20 min at 20-25°C (68-77°F) and filter the supernatant if necessary.

↓

Dilute ***Sample Extract*** 20-fold with ***Diluent I***
(If necessary, dilute further using ***Diluent II***)

(ELISA procedure)

Attach the ***Antibody-coated Microplate Module (A)*** to the mounting frame

Pipette 100 µL ***Working Peanut Standard*** and ***Working Sample Solution*** into each well

↓

-- 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 ***Enzyme-conjugated Antibody (C)*** per 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 ***Enzyme Substrate (D)*** per well

↓

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

↓

Stop the enzyme reaction by adding 100 µL ***Stop Solution (E)*** per well

↓

Measure absorbance at 450 nm
(Subtract the reading at a reference wavelength: any wavelength from 600 to 650 nm, inclusive)

↓

Calculate the concentration of peanut protein using the standard curve

* The wells are emptied completely by inverting and tapping the microplate on a clean paper towel.

Results and Interpretation

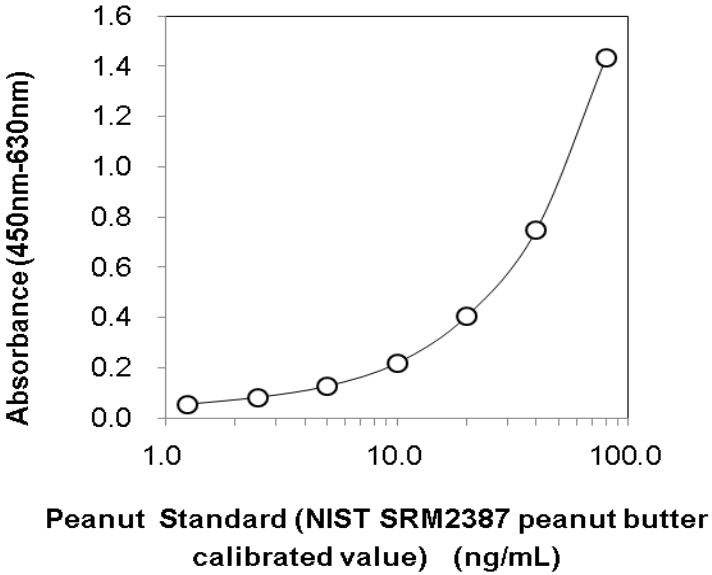
1. Determine the mean absorbance for each set of ***Working Sample Solution*** and ***Working Peanut Standard***. Use graphing software, the built-in graphing feature of the microplate reader, or graph paper to construct a standard curve by plotting the mean absorbance value for each standard on the Y axis versus the corresponding ***Working Peanut Standard*** concentration on the X axis.
   Note*: A standard curve should be generated for each assay.*
2. The peanut 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 ***Working Sample Solution*** is greater than the absorbance of the 80 ng/mL standard, increase the dilution of ***Working Sample Solution*** to 40-fold or greater as appropriate instead of 20-fold, and then assay again.

The peanut protein content in a sample, in µg/g, can be estimated using the following formula:

Peanut protein content (µg/g) = OV× Dilution A× Dilution B× 1/1,000

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

Typical Standard Curve



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 20-25°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. Do not use the kit after the expiration date indicated on the outside box.

WARNINGS AND PRECAUTIONS

(General)

1. Peanut protein is used in this kit. Users who are allergic to peanut 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 use 2-mercaptoethanol which is categorized as hazardous substance (CAS No.60-24-2), therefore perform the assay under the well-ventilated hood, and handle the reagents cautiously.
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.

(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 peanut proteins. Therefore, food samples that give a negative result may still contain peanut material which is either unreactive or present at concentrations below the limit of detection. It should not be assumed that such foods are peanut 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	***Peanut Standard (Base Protocol)***	Equilibrate to 20-25°C (68-77°F)
	Working Peanut Standards (0.78, 1.56, 3.13, 6.25, 12.5 and 25 ng/mL)	Dilute ***Peanut Standard*** (50 ng/mL) with ***Diluent II***, sequentially
B	***Peanut Standard (Alternate Protocol)***	Equilibrate to 20-25°C (68-77°F)
	Working Peanut Standards (1.25, 2.5, 5, 10, 20, 40 and 80 ng/mL)	Dilute ***Peanut Standard*** (160 ng/mL) with ***Diluent II***, sequentially
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

TECHNICAL ASSISTANCE

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

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WARRANTY

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