

**[For food inspection]****FASTKIT ELISA Ver. II Soybean <<Instruction Manual>>**

* Please read this manual carefully before using the kit.

Cat# NPH-999100425-EX

- Development history and characteristics -

The Food Sanitation Law requires food manufacturers to indicate designated raw materials considered likely to induce food allergy including egg, milk, wheat, buckwheat, and peanut on the label of food products containing them. The law recommends that soybean should be also indicated on product labels as a material similar to the designated raw materials. Since soybean is contained various food products and relatively frequently induces allergy among materials for which the indication on the product label is recommended, it is important to determine the content of soybean-derived proteins as for the five designated raw materials and exclude raw material-derived carry-over or unexpected contamination during the manufacturing process.

This product is an enzyme-linked immunosorbent assay-based measurement kit containing reagents required for measuring soybean proteins in food products. This kit can measure soybean-derived proteins in food products in a simple operation.

[Characteristics]

- 1) This kit can measure soybean-derived proteins in food products with high sensitivity.
- 2) The kit can be applied to a wide variety of samples from raw materials to processed foods.

- Contents of kit -

A: Antibody immobilized plate (with cover)	96 wells (8 wells × 12 strips) × 1
B: Standard solution (50ng/mL) < manufactured by Oriental Yeast Co.,Ltd >	1.8 mL × 1
C: Dilution buffer	100 mL × 1
D: Biotin-labeled antibody	150 μL × 1
E: Enzyme (peroxidase)-labeled streptavidin	150 μL × 1
F: Chromogenic substrate (TMB)	12 mL × 1
G: Stop solution (0.5 N H ₂ SO ₄)	12 mL × 1
H: Concentrated washing solution (1/10 concentration)	100 mL × 1
I: Extraction reagent ① (1/20 concentration)	50 mL × 1
J: <u>Extraction reagent for soybean ② (1/10 concentration)</u>	<u>100 mL × 1</u>
K: Extraction reagent ③ (1/20 concentration)	50 mL × 1
L: Instruction manual	1

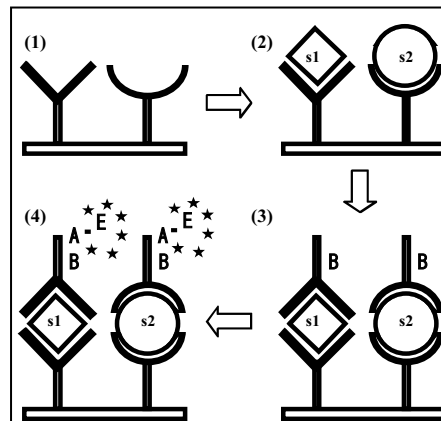
Note: The concentration of the Extraction reagent for soybean ② is different from that of the Extraction reagent ② for other items (egg, milk, wheat, buckwheat, and peanut).

- Objective / performance -

- This kit can measure soybean derived proteins in food products in a range of 1 to 20 ppm.

- Measurement principle -

- (1) An antibody to bind with more than one soybean-derived protein has been immobilized in the plate wells.
- (2) The immobilized antibody captures soybean proteins (s1, s2...) in the measurement solution to form complexes.
- (3) The Biotin-labeled antibody binds to the complexes to form sandwich complexes.
- (4) The Enzyme-labeled streptavidin then binds to the sandwich complexes through the reaction between biotin and streptavidin. The enzymatic substrate (TMB) is then added to produce a color.

**- Necessary apparatuses -****[Preparation of reagents]**

- Measuring cylinders, beakers, and micropipettes

[Preparation of measurement solution]

- Grinding machine (food cutter), balance, 50-mL plastic centrifugal tubes (with cap), shaking machine, centrifugal machine (A centrifugal machine of 3,000 × g or higher and with an ability of centrifugation at room temperature is recommended), filter paper, and funnel

[Measurement operation and data analysis]

- Micropipette, test tubes or micro tubes, blotting paper (paper towel), micro-plate reader (with filters with a wavelength of 450 nm and 600 to 650 nm), and analytical software (that can use the 4-parameter analysis)



- Preparation of reagents -

[Reagents to be used as supplied]

- Dilution buffer: Let it warm to room temperature (20 to 25°C) before use.
- Chromogenic substrate: Collect a necessary amount into a light-resistant container and let it warm to room temperature (20 to 25°C) before use.
- Stop solution: Let it warm to room temperature (20 to 25°C) before use.

[Reagents to be prepared before use]

- Sample extracting medium for soybean: Mix the Extraction reagent ①, Extraction reagent for soybean ②, Extraction reagent ③, and purified water at a ratio of 1:2:1:16 and stir it well before use.
- Diluent for reference standard: Prepare a 1/20 dilution of the sample extracting medium for soybean using the Dilution buffer.
- Standard solution: Dilute the standard solution with the diluent for the reference standard with reference to the following dilution examples.

<Example of preparing sample extracting medium for soybean: preparation for 24 samples>

Extraction reagent ① (1/20 concentration)	: 25 mL
Extraction reagent for soybean ② (1/10 concentration)	: 50 mL
Extraction reagent ③ (1/20 concentration)	: 25 mL
Purified water	: 400 mL
Total	: 500 mL

<Examples of dilution of standard solution>

Concentration of reference standard (ng/mL)	50	25	12.5	6.25	3.125	1.5625	0.78125	0
Amount of standard solution to be added (μL)	800	400	400	400	400	400	400	0
Amount of diluent for reference standard to be added (μL)	0	400	400	400	400	400	400	400

- Concentrated washing solution: Prepare a 1/10 dilution of the concentrated washing solution using purified water.
- Biotin-labeled antibody: Prepare a 1/100 dilution of the Biotin-labeled antibody using the Dilution buffer that was let warm to room temperature beforehand. Use the dilution within 15 minutes.
- Enzyme-labeled streptavidin: Prepare a 1/100 dilution of the Enzyme-labeled streptavidin using the Dilution buffer that was let warm to room temperature beforehand. Use the dilution within 15 minutes.

<Diluents to be used and objects to be diluted>

Objects to be diluted	Standard solution	Supernatant from extraction or filtrate	Biotin-labeled antibody	Enzyme-labeled streptavidin
Dilution buffer				
Diluent for reference standard	○			

Note 1) Keep the standard solution, Biotin-labeled antibody, and Enzyme-labeled streptavidin in a refrigerator until immediately before and immediately after use.

Note 2) Do not mix or replace the same reagent from different lots.

Note 3) Do not use the reagents for which the designated measurement parameter is specified on the label (Antibody immobilized plate, standard solution, Biotin-labeled antibody, Enzyme-labeled streptavidin, and extraction reagent for soybean ②) for other measurement parameters. Reagents without designated measurement parameter may be used for all measurement parameters.

Note 4) Accurately perform pipetting including dispensing and diluting because it greatly influences measurement accuracy. Replace the micropipette tip at every dispensing or diluting operation.

Note 5) Although the extraction reagent for soybean ② may become solidified during storage or distribution, the change does not indicate any loss of performance. Warm it by putting it in warm water before use to melt the precipitates.

Note 6) The extraction reagent for soybean ② in this kit is different from the extraction reagent ② for other measurement items (egg, milk, wheat, buckwheat, and peanut).

- Extraction operation (Example of operation for a general food product) -

- 1) Uniformly crush a package unit of a food sample (test sample) using a Grinding machine to a prepared sample (Note 1).
- 2) Weigh one gram of the prepared sample into a plastic centrifugal tube. Add 19 mL of the sample extracting medium for soybean into the tube. Mix them well to prepare a uniform dispersion (Note 2).
- 3) Set the centrifugal tube horizontally in a shaking machine and shake it overnight (at least 12 hours) at room temperature to obtain an extract (Notes 3 and 4).
- 4) Centrifuge the extract at 3,000 × g and room temperature for 20 minutes to collect the supernatant. If no sediment occurs, pass the centrifuged extract through a filter (Note 5).
- 5) Prepare a 1/20 dilution of the collected supernatant or filtrate using the Dilution buffer and use it as a measurement solution (Note 6).

Note 1) To prevent contamination by experimental apparatuses, use only well-cleaned or disposable apparatuses. Thoroughly clean the Grinding machine or homogenizer every time it is used (clean it using a neutral detergent and immerse it in an alkaline detergent solution overnight or ultrasonically clean it in an alkaline detergent solution for at least 30 minutes).

Note 2) Fully disperse the sample using a vortex mixer, taking care to prevent bubbling.

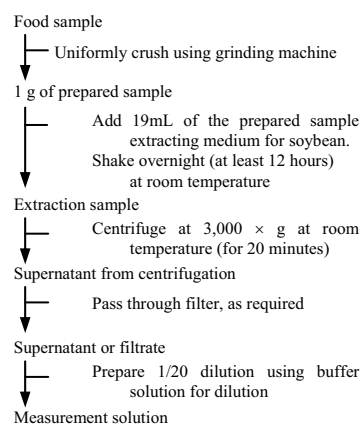
Note 3) Set the shaking cycle at 90 to 110 cycles per minute and the shaking width at about three centimeters so that the solution in the tube will hit both ends of the tube during shaking. Occasionally turn the centrifugal tube upside down to prevent the sample from adhering to the tube at the fluid level.

Note 4) Determine pH of the extract and, if necessary, adjust it to a neutral area (pH 6.0 to 8.0).

Note 5) Keep the collection volume of the supernatant as constant as possible. Remove the oil layer, if possible. It is recommended that filtration should be performed to obtain accurate results.

Note 6) Keep the collected supernatant or filtrate at 4°C and use it for measurement within one week. It is recommended that the supernatant or filtrate should be cryopreserved if it is to be kept for one week or longer. The allowable storage period under refrigeration or cryopreservation depends on the ingredients of the food product.

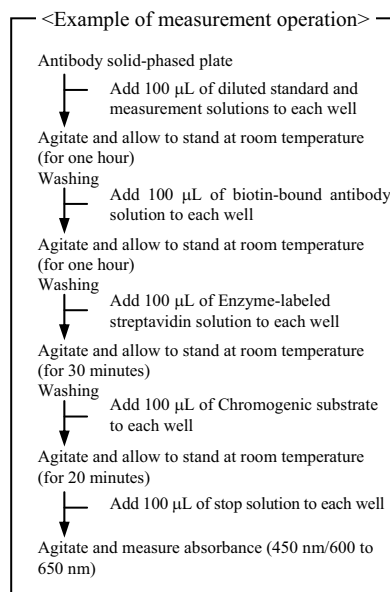
<Example of extraction operation>





- Measurement operation -

- 1) Let the Antibody immobilized plate warm to room temperature in the original aluminum pouch. Remove it from the pouch immediately before use (Note 1).
- 2) Add 100 μ L each of the diluted standard and measurement solutions into the wells (Note 2).
- 3) Lightly agitate the solution in the wells and allow the plate to stand for one hour at room temperature (20 to 25°C) for reaction.
- 4) After the reaction is completed, discard the standard and measurement solutions. Add 250 μ L of the diluted washing solution to each well and discard it, and repeat this procedure five times (Note 3).
- 5) Add 100 μ L of the prepared Biotin-labeled antibody solution to each well.
- 6) Lightly agitate the solution in the wells and allow the plate to stand for one hour at room temperature (20 to 25°C) for reaction.
- 7) After the reaction is completed, discard the Biotin-labeled antibody solution and wash the plate as described in the above Step 4.
- 8) Add 100 μ L each of the prepared Enzyme-labeled streptavidin solution to each well.
- 9) Lightly agitate the solution in the wells and allow the plate to stand for 30 minutes at room temperature (20 to 25°C) for reaction.
- 10) After the reaction is completed, discard the Enzyme-labeled streptavidin solution and wash the plate as described in the above Step 4.
- 11) Let the Chromogenic substrate warm to room temperature beforehand and add 100 μ L of the reagent to each well.
- 12) Lightly agitate the reagent in the wells and allow the plate to stand for 20 minutes at room temperature (20 to 25°C) to make the reagent produce a color. It is recommended that this step should be performed under a light-shielded condition to increase data reproducibility.
- 13) Let the stop solution warm to room temperature beforehand and add 100 μ L of the solution to each well. Lightly agitate the solution to stop color development (Note 4).
- 14) After agitation, measure the absorbance at the primary wavelength of 450 nm and secondary wavelength of 600 to 650 nm using a plate reader.



- Note 1) When a part of the plate is used, return the unused strips into the aluminum pouch with a desiccant and keep the pouch in a refrigerator.
- Note 2) It is recommended that three wells each should be used for the measurement of the standard and measurement solutions. Make sure to prepare a standard curve based on the measurements of the standard solution for every measurement.
- Note 3) The cleaning operation is critical for accurate measurement. Completely remove the solution and air bubbles in the wells by holding the plate upside down and strongly tapping it onto a power towel several times, and then add the next reagent immediately.
- Note 4) Handle the stop solution carefully because it contains 0.5 N sulfuric acid

- Data analysis -

- 1) Prepare a standard curve graph from the absorbance values of the standard solution using the 4-parameter analysis (Note 1).
- 2) Read soybean protein concentration in the measurement solution (ng/mL) from the standard curve.
- 3) Multiply the soybean protein concentration or corrected soybean protein concentration by the dilution factor used during the extraction operation (400) to calculate the soybean protein concentration in the food product.

- Note 1) The measurement results may vary when the standard curve graph is prepared using an analytical technique other than the 4-parameter analysis.

- Performance -

[Test method]

This product was confirmed to comply with the following acceptance criteria when it was tested according to the "Guidelines for Evaluating Test Methods for Food Products Containing Allergic Substances" as specified in the PFSB/DFS Notification No. 1106001 from the Director of the Dept. of Food Safety, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare dated November 6, 2002, "Test methods for food products containing allergic substances" (partially amended according to the PFSB/DFS Notification No. 1011002 dated October 11, 2005, PFSB/DFS Notification No. 0324001 dated March 24, 2006, and PFSB/DFS Notification No. 0622003 dated June 22, 2006 from the director of the same department).

[Recovery rate]

The inspection method shall have a recovery rate of more than 50% and less than 150% in the interlaboratory validation with at least eight laboratories using at least five samples (each containing 10 μ g/g of a soybean protein).

[Reproducibility]

The inspection method shall have reproducibility of less than 25% in the interlaboratory validation with at least eight laboratories using at least five samples (each containing 10 μ g/g of a soybean protein).



- False positive/false negative -

- 1) No cross reactivity has been observed with the five designated raw materials (egg, milk, wheat, buckwheat, and peanut).
- 2) The test at our laboratory confirmed that the kit produced a false positive result for cowpea. For the other false positive results, refer to the "List of food products producing false positive or false negative results" on the homepage of Nippon Meat Packers, Inc. R&D Center on the web.
- 3) The kit may cause a non-specific reaction under the presence of a very high concentration of a protein. In such a case, appropriately dilute the sample and repeat the test. Always use the diluent for the reference standard when a 1/20 dilution of a sample is further diluted.
- 4) The kit may produce a false-negative result for the following substances because they contain a limited amount of soybean proteins or their soybean proteins have been decomposed during the manufacturing process: soysauce, soybean oil, some fermented soybeans, some soybean peptides, some soybean isoflavones, and some soybean lecithins.

- Precautions for use or handling -

[General precautions]

- 1) Read this instruction manual carefully and observe the operation procedures.
- 2) Do not use the kit or its component after its expiration date. The expiration date is indicated on the outer package and each component's label.
- 3) This kit is intended for measuring soybean proteins in food products and not for making a diagnosis of food allergy to soybean. The correlation between the measurement results of the kit and development of allergic symptoms has not been established.
- 4) Make a comprehensive decision on the presence/absence of soybean proteins by examining not only the result of the present kit, but also other data including raw materials and manufacturing records.
- 5) For how to use the machines and apparatuses used with the kit, refer to the instruction manuals provided by their manufacturers/distributors.
- 6) This instruction manual is intended to serve as a guideline for persons in charge of inspection. Verify the validity of the application of each inspection step to each food product.
- 7) The specifications of this kit are subject to change without prior notice.

[Safety precautions]

- 1) Take care to prevent the exposure of the skin, mucosal membrane, or clothes to the reagents of the kit.
- 2) When any of the reagents accidentally enters the eye or mouth, immediately take appropriate first-aid treatment, such as rinsing with tap water, and receive medical attention.

[Precautions for disposal]

- 1) Observe local regulations to dispose of the kit, samples, and measurement solution, etc. with full attention to the possible effect on sanitation and environment.

- Storage and expiration date -

- 1) Storage: Keep this kit under a refrigerated (2 to 8°C) and light-shielded condition. Avoid freezing the kit.
- 2) Expiration date: Six months from the date of manufacture: The expiration date is indicated on the outer package and each component's label.

- References -

- 1) PFSB/DFS Notification No. 1106001 from the Director of the Dept. of Food Safety, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, "Test methods for food products containing allergic substances" (partially amended according to the PFSB/DFS Notification No. 1011002, PFSB/DFS Notification No. 0324001, and PFSB/DFS Notification No. 0622003)
- 2) Imai T. and Ebisawa M.: The 2002 Scientific Research Report of Ministry of Health, Labour, and Welfare
- 3) Marui E.: Food Sanitation Research, Vol. 52 (5), 25-31, 2002
- 3) Akiyama H. and Toyoda M.: Food Sanitation Research Vol. 52 (6), 65-73, 2002

Manufactured by Nippon Meat Packers, Inc. R&D Center



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