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**[For food inspection]**

# FASTKIT ELISA Ver. III Soybean <<Instruction Manual>>

Please read this manual carefully before using the kit.

## - Development history and characteristics –

This product is a food allergen screening test kit which can detect soybean proteins in foods.The Food Sanitation Act requires food manufacturers to indicate designated raw materials considered likely to induce food allergy including egg, milk, wheat, buckwheat, peanut, shrimp, and crab on the labelling of food products containing them. The act recommends that soybean should be also indicated on the labelling of food products containing it.

This product has been developed based on the guidelines shown by CAA Notification No. 286 from the Deputy Secretary General of the Consumer Affairs Agency dated September 10, 2010, "Test methods for food products containing allergic substances". This product can measure soybean proteins in the food such as raw materials and processed foods widely.

This is the improved kit by removing 2-mercaptoethanol from "FASTKIT ELISA Ver. Ⅱ Soyben", and not including poisonous substance. This product can keep and dispose like a general reagent.

Further, Extraction reagent ② has been common with the other items of "FASTKIT ELISA Ver.Ⅲ series" by this improvement. The prepared Sample extracting medium can use in the other items of "FASTKIT ELISA Ver.Ⅲ series".

## - Kit contents-

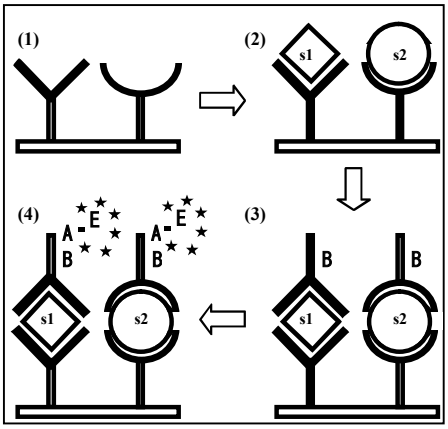
| | |
|---|---|
| A: Antibody immobilized plate (with cover) | 96 wells (8 wells × 12 strips) × 1 |
| B: Standard solution (50 ng/mL) | 1.8 mL × 1 |
| C: Dilution buffer | 100 mL × 1 |
| D: Biotin-labeled antibody | 150 µL × 1 |
| E: Enzyme (peroxidase)-labeled streptavidin | 150 µL × 1 |
| F: Chromogenic substrate | 12 mL × 1 |
| G: Stop solution (0.5 N $H_2SO_4$) | 12 mL × 1 |
| H: Concentrated washing solution (10× concentration) | 100 mL × 1 |
| I: Extraction reagent ① (20× concentration) | 50 mL × 1 |
| J: Extraction reagent ② (20× concentration) | 50 mL × 1 |
| K: Extraction reagent ③ (20× concentration) | 50 mL × 1 |
| L: Instruction manual | 1 |

## - Objective/performance -

- This kit is intended to measure soybean proteins in food products.
- This kit can measure standard soybean proteins in a solution at a concentration ranging from 0.78 to 50 ng/mL.

## - Measurement principle -

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




## - Necessary apparatuses -

**[Preparation of reagents]**

- Measuring cylinders, beakers, micropipettes, and disposable tips

**[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, disposable tips, 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)

Note 1) To prevent the contamination by experimental instruments, use disposable apparatuses or monopolized instruments as much as possible. If you use non-monopolized instruments, wash them using an alkaline detergent to remove proteins before use.

## - Reagent preparation -

**[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: Prepare a sample extracting medium by thoroughly shaking a mixture consisting of the Extraction reagents ①, ②, ③ and purified water at a ratio of 1:1:1:17 before use.
- Diluent for reference standard: Prepare a 1/20 dilution of the sample extracting medium with 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: preparation for 24 samples>

| | |
|---|---|
| Extraction reagent ① (20× concentration) | : 25 mL |
| Extraction reagent ② (20× concentration) | : 25 mL |
| Extraction reagent ③ (20× concentration) | : 25 mL |
| Purified water | : 425 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, and Enzyme-labeled streptavidin) for other measurement parameters. Reagents without designated measurement parameter may be used for all measurement parameters.
Note 4) Although the extraction reagent ②・③ may become cloudy and form precipitates during storage or distribution, the changes do not indicate any loss of performance. Warm it by putting it in warm water before use to melt the precipitates.

## - 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 prepareed sample
2) Weigh one gram of the prepared sample into a plastic centrifugal tube. Add 19 mL of the Sample extracting medium into the tube. Mix them well to prepare a uniform dispersion (Note 1).
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 (Note 2 and 3).
4) Centrifuge the extract at 3,000 × g for 20 minutes to collect the supernatant. If no sediment occurs, pass the centrifuged extract through a filter (Note 4).
5) Prepare a 1/20 dilution of the collected supernatant or filtrate using the Dilution buffer and use it as a measurement solution.

<Example of extraction operation>

Food sample
↓ Uniformly crush using grinding machine
1 g of prepared sample
↓ Mix with 19 mL of prepared sample extracting medium
Shake overnight (at least 12 hours) at room temperature
Extraction sample
↓ Centrifuge at 3,000 × g at room temperature (for 20 minutes)
Supernatant from centrifugation
↓ Pass through filter, as required
Supernatant or filtrate
↓ Prepare 1/20 dilution using the Dulution buffer
Measurement solution

Note 1) Fully disperse the sample using a vortex mixer, taking care to prevent bubbling.
Note 2) 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 3) Determine pH of the extract and, if necessary, adjust it to a neutral area (pH 6.0 to 8.0).
Note 4) 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.

## - 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 µL 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 µL of the diluted washing solution to each well and discard it, and repeat this procedure five times (Note 3).
5) Add 100 µL 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 µL 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 µL 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 µL 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.

<Example of measurement operation>

Antibody immobilized plate
↓ Add 100 µL of diluted standard and measurement solutions to each well
Agitate and allow to stand at room temperature (for one hour)
Washing
↓ Add 100 µL of biotin-bound antibody solution to each well
Agitate and allow to stand at room temperature (for one hour)
Washing
↓ Add 100 µL of Enzyme-labeled streptavidin solution to each well
Agitate and allow to stand at room temperature (for 30 minutes)
Washing
↓ Add 100 µL of Chromogenic substrate to each well
Agitate and allow to stand at room temperature (for 20 minutes)
↓ Add 100 µL of stop solution to each well
Agitate and measure absorbance (450 nm/600 to 650 nm)

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 the soybean protein concentration in the measurement solution (ng/mL) from the standard curve.
3) Multiply the soybean protein concentration by the dilution factor 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 the 4-parameter analysis.

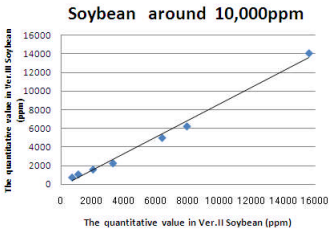
## - Performance of this kit-

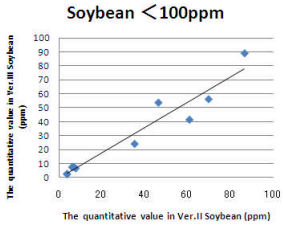
"FASTKIT ELISA Ver.Ⅲ Soybean" was evaluted based on " Guidelines for Evaluating Improved Methods of the Test Methods for Food Products Containing Allergic Substances". Result of FASTKIT ELISA Ver.Ⅱ Soybean was taken to the X-axis, and result of FASTKIT ELISA Ver.Ⅲ Soybean was taken to the Y-axis, and the quantitive values of them were plotted. The results are shown below. These results indicated that FASTKIT ELISA Ver.Ⅲ Soybean was satisfied to the criteia of the guideline (slope:0.75-1.25, correlation coefficient: R＜0.9).

[Samples]

| around 10,000ppm | ＜100 ppm |
|---|---|
| Bread 1 | Bread 3 |
| Bread 2 | Bread 4 |
| Confectionery 1 | Bread 5 |
| Sausage1 | Confectionery 2 |
| Sausage2 | Confectionery 3 |
| Bacon | Confectionery 4 |
| Rice seasoning | Confectionery 5 |
| Butter | Sausage3 |
| Yoghurt | *Surimi*2 |
| *Surimi*1 | Noodle |

[Results]



## - False positive/false negative -

1) No cross reactivity has been observed with the specific allergenic ingredients (egg, milk, wheat, buckwheat, peanut, shrimp, and crab).
2) For more information of false positive or false negative results, refer to the "List of food products producing false positive or false negative results" on the homepage of NH Foods Ltd. 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.

## - 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. 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) The present instruction manual is intended to serve as guidelines for those in charge of food inspection. Verify the validity of the application of each inspection step to each food product.
7) Buyer assumes all risk and liability resulting from the use of this product. NH Foods Ltd. shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.
8) The specifications of this kit are subject to change without prior notice.

## - 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) CAA Notification No. 286 from the Deputy Secretary General of the Consumer Affairs Agency, "Test methods for food products containing allergic substances"
2) 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
4) CAA Notification No. 36 from the Deputy Secretary General of the Consumer Affairs Agency, "Partial Revision of "Test methods for food products containing allergic substances""

Manufactured by NH foods Ltd. R&D Center

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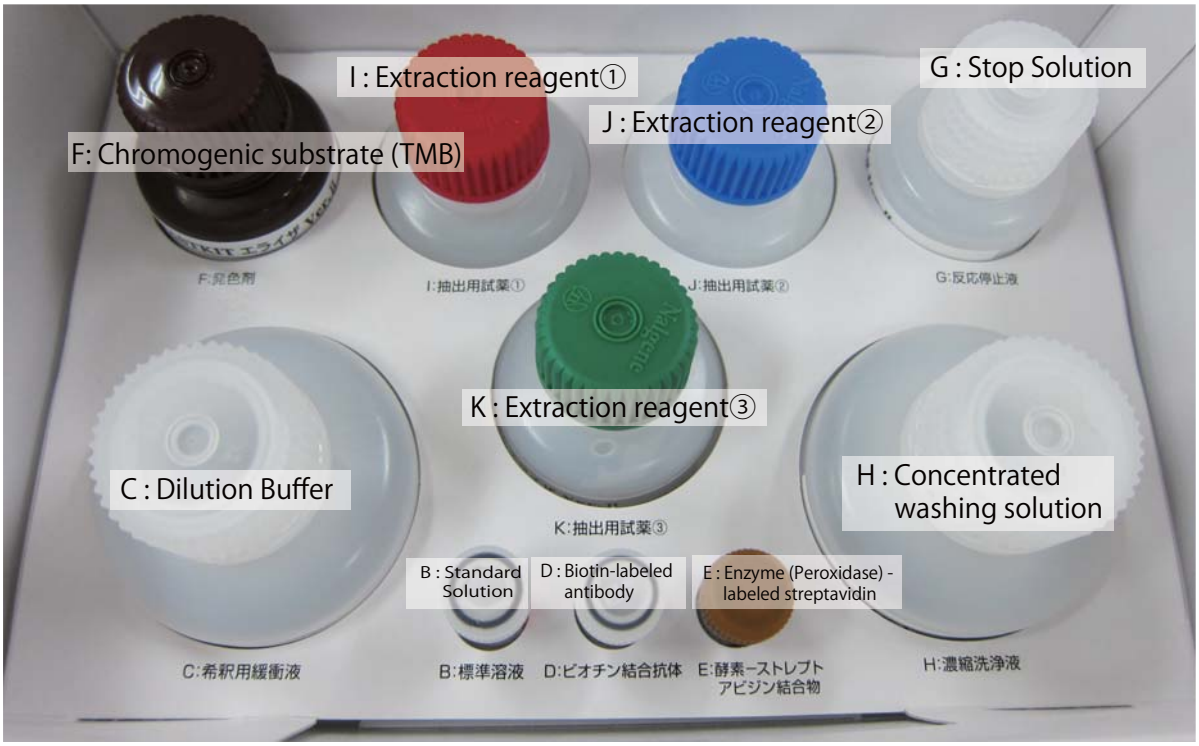
I : Extraction reagent①
G : Stop Solution
J : Extraction reagent②
F: Chromogenic substrate (TMB)
F:発色剤
I:抽出用試薬①
J:抽出用試薬②
G:反応停止液
K : Extraction reagent③
C : Dilution Buffer
H : Concentrated washing solution
K:抽出用試薬③
B : Standard Solution
D : Biotin-labeled antibody
E : Enzyme (Peroxidase) - labeled streptavidin
C:希釈用緩衝液
B:標準溶液
D:ビオチン結合抗体
E:酵素−ストレプトアビジン結合物
H:濃縮洗浄液