

For the detection of allergen in foods

Allergeneye Immunochromatography

**Egg, Milk (Casein), Milk (Whey), Wheat,
Buckwheat, Peanut, Crustacean, Soy, Sesame**

Instruction Manual

This kit is an immunochromatography kit which uses monoclonal antibodies that are highly specific to certain proteins originating from the food allergen to be detected. It is capable of detecting food allergens contained in a wide range of food products from raw materials to processed food products. It can also be used to test swab samples, and rinse water.

CAUTION!

1. Do not attempt to drink the kit reagent, touch it with bare hands or allow it to splash into eyes.
2. Read this Manual well before testing and conduct tests properly according to the operation procedures.

This kit contains research reagents for detecting food allergens in test samples, not clinical test drugs to diagnose the presence of food allergy onset and so forth. There are individual differences in onset of allergy, and correlation between the quantity of allergen intake and allergy onset is unknown.

Contents of the kit

Packaging unit: 20 tests

	Name	20 tests
A	Test strips (individual packaging in transparent plastic bag, with a plate-shaped desiccant)	20 strips
B	Detection reagent (5 tubes/1 bag package, freeze-dried product)	20 tubes (4 bags)
C	Specimen extraction solution	
	Extraction solution (1) (10-time concentrated)	50 mL x 1
	Extraction solution (2) (10-time concentrated)	50 mL x 1
D	Instruction Manual (this booklet)	1 copy
E	Detection reagent tube stand (10 holes, made of paper)	2 pieces

Types of kits

Name of kit	Product code	Protein to be detected	Label color
Allergeneye Immunochromatography Egg	61950	Ovalbumin	Yellow
Allergeneye Immunochromatography Milk (Casein)	61951	α 1-casein	Blue
Allergeneye Immunochromatography Milk (Whey)	61952	β -lactoglobulin	Purple
Allergeneye Immunochromatography Wheat	61953	Gliadin	Red
Allergeneye Immunochromatography Buckwheat	61954	24 kDa protein	Green
Allergeneye Immunochromatography Peanut	61955	Ara h 1	Brown
Allergeneye Immunochromatography Crustacean	61956	Tropomyosin	Orange
Allergeneye Immunochromatography Soy	61957	7S globulin	Light blue
Allergeneye Immunochromatography Sesame	61958	11S globulin	Black

Performance

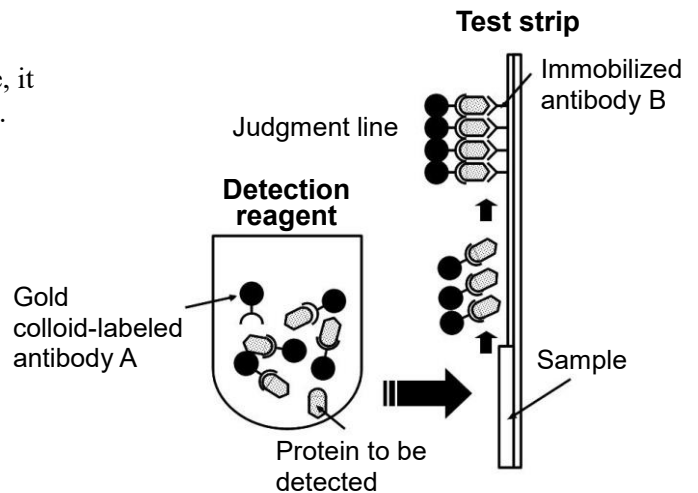
It indicates the positive sign when the concentration of protein originating from the food allergen contained in the tested food product is 2 ppm or higher.

Method of storage/expiration date

1. Method of storage: Store this product at room temperature (1 to 30°C) while avoiding high temperature and high humidity.
2. Expiration date: Indicated on the outer box, transparent plastic bags and so forth.

Principle

1. Add the test sample to the detection reagent.
2. If the protein to be detected is contained in test sample, it forms a complex with gold colloid-labeled antibody A.
3. When the test strip is soaked in this solution, this complex moves over the test strip, captured by immobilized antibody B, resulting in visual observation of the purplish red judgment line.
4. If the protein to be detected is not contained, it does not form a complex and thus the judgment line is not observed.



Required Materials (not included in the kit)

The materials listed here are examples. There are other materials with equivalent performance and materials that can be substituted. Please contact us if you have any questions.

A: Food product testing	B: Swab testing
<ul style="list-style-type: none"> • Graduated cylinder • Polypropylene centrifuge tube (50 mL) • Water bath (for heating at approx. 100°C) • Purified water • Filter paper (ADVANTEC No.5A, etc.) • Timer • Tube stand • Test tube mixer 	<ul style="list-style-type: none"> • Graduated cylinder • Polypropylene centrifuge tube (50 mL) • Water bath (for heating at approx. 100°C) • Purified water • Filter paper (ADVANTEC No.5A, etc.) • Timer • Tube stand • Test tube mixer
<ul style="list-style-type: none"> • Micropipettor (one capable of fractionating 100 µL) • Food processor or mixer • Measuring instrument 	<ul style="list-style-type: none"> • Micropipettor (one capable of fractionating <u>1 mL and 100 µL</u>) • <u>Commercial swab tool*</u> (containing <u>10 mL phosphate-buffered saline</u>)

* Commercial products for microbial testing can be used as the swab tools. However, make sure to use ones that do not contain culture medium components such as peptone or proteins including allergens.

Operation procedure 1: Preparation of test sample

1. Preparation of the specimen extraction solution

(1) Be sure to allow the kit to return to 20 to 25°C before using.

* Let test strips and detection reagent return to the room temperature without taking them out of the bags.

(2) Prepare “C: Specimen extraction solution.”

* Add 1 unit volume each of “extraction solution (1) (10-time concentrated)” and “extraction solution (2) (10-time concentrated)” to 8 unit volumes of purified water, and stir the mixture well.

E.g.) When preparing 20 mL:

Add 2 mL of “extraction solution (1) (10-time concentrated)” and 2 mL of “extraction solution (2) (10-time concentrated)” to 16 mL of purified water.

* Dilute with care not to let it foam, as it easily foams.

* “Extraction solution (1)” may form precipitation during storage. In this case, put the entire container in a warm water bath to dissolve the precipitation completely. Use it for testing after letting it cool to around the room temperature if it has been warmed in warm water.

2. Preparation of test sample (dilution operation is necessary in (5) only for wheat.)

A: Food product testing		B: Swab testing	
(1)	Pulverize and mix the test sample in a food processor, mixer and so forth so that it becomes uniform.	(1)	Swab the area to be tested with a swab tool. * Determine the area to be swabbed upon verification. (E.g.: A 10 cm x 10 cm area) * Return the swab part that swabbed the area into the container and mix well by shaking to dissolve the swabbed sample.
(2)	Measure and collect 1 g of test sample which was made uniform into a 50-mL centrifuge tube and add 19 mL of specimen extraction solution.	(2)	Measure and collect 1 mL of the solution in (1) into a 50-mL centrifuge tube and add 19 mL of specimen extraction solution.
(3)	Stir well, then heat it in a boiling water bath for 10 minutes. Cool the sample to around the room temperature under running water and so forth after heating. * Acidic or alkaline food products and so forth may change in pH and no longer be around neutral (pH 6.0 to 8.0) after extraction. In this case, check the pH and adjust it so that it stays around neutral (pH 6.0 to 8.0) as necessary.	(3)	Stir well, then heat it in a boiling water bath for 10 minutes. Cool the sample to around the room temperature under running water and so forth after heating.
(4)	Filter the solution in (3) with a filter paper. * If filtrate collection is difficult, conduct centrifugation (such as 3,000 x g for 20 minutes), collect the supernatant and filter it with a filter paper. * Make sure that the sample temperature is room temperature (20°C to 25°C) when conducting centrifugation.	(4)	If high levels of food ingredients are contained in the solution in (3), filter it with a filter paper.
(5)	[For samples other than wheat] Conduct testing while using the filtrate obtained in (4) as the test sample. [For wheat] Conduct testing while using the filtrate obtained in (4) which is then diluted to 5 times with specimen extraction solution as the test sample.	(5)	[For samples other than wheat] Conduct testing while using the solution in (3) or the filtrate obtained in (4) as the test sample. [For wheat] Conduct testing while using the solution in (3) or the filtrate obtained in (4) which is then diluted to 5 times with specimen extraction solution as the test sample.

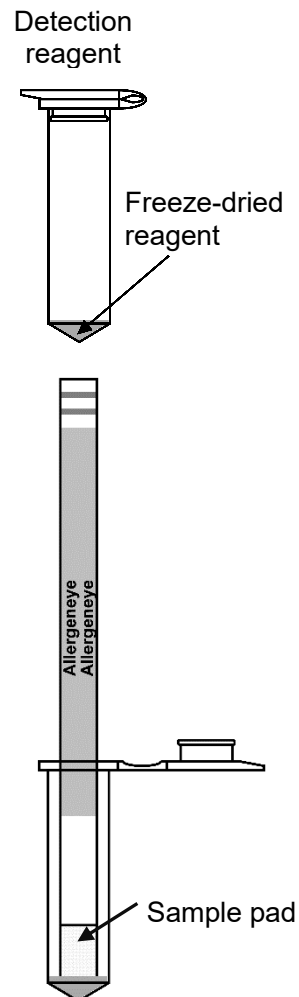
Operation procedure 2: Testing with test strips

Use after allowing them to return to room temperature in the bags if test strips and detection reagent are stored under refrigeration.

* Note: Conduct the operations (1) to (4) within 15 minutes.

(1) Take the detection reagent out of the aluminum bag with fastener.

- * Take the detection reagent out of the bag immediately before using and close the fastener tightly after taking it out.
- * The aluminum bag in which detection reagent is stored can be heat-sealed. To store for a long period, it is recommended that it is sealed tightly with a heat sealer.
- * If the fastener is not closed tightly, the detection reagent will absorb moisture and become solidified. It may not be possible to test properly using detection reagent which has absorbed moisture. Use only the detection reagent that is in an appropriate condition.

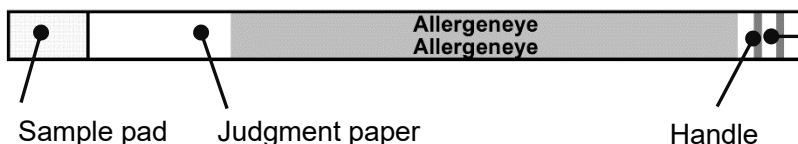


(2) Open the lid of the detection reagent, add 100 μ L of test sample to mix well by shaking and dissolve the freeze-dried reagent uniformly, and set it on the detection reagent tube stand.

- * Add the sample after scraping it off if the freeze-dried reagent is adhering to the tube lid or wall surface.
- * Although the shape of the freeze-dried reagent may vary between tubes, use it as it is, since the quantity of reagent is uniform.

(3) Take a test strip out of the transparent plastic bag.

- * Take the test strip out of the bag immediately before use.
- * Since the judgment paper part is not protected by any film, hold the handle part indicated by 2 lines, and handle carefully not to touch the judgment paper part.



Colors of the lines (2)
Yellow (Egg), blue (Casein),
purple (Whey), red (Wheat),
green (Buckwheat), brown (Peanut),
orange (Crustacean), light blue (Soy),
black (Sesame)

(4) Insert the test strip into the tube in (2), and leave it standing.

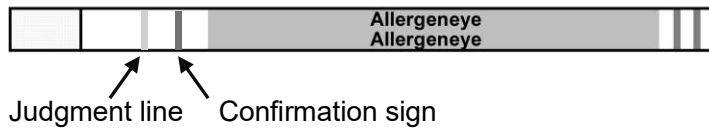
- * Make sure to insert so that the end of the sample pad reaches the bottom of the tube.

(5) Remove the test strip after 20 minutes to judge the result.

- * Judge the result after checking that the pink confirmation sign has become visible.
- * Judge the result 20 minutes after inserting the test strip into the tube, and not any later.

Judgment

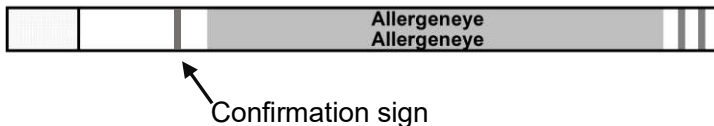
- Positive: 2 lines, which are the purplish red judgment line and the pink confirmation sign, are both visible.



Protein originating from food allergen is contained in the tested food product or swabbing solution at 2 ppm or higher.

* Consider it positive as long as the purplish red judgment line is visible, regardless of the depth of the line color.

- Negative: Only the pink confirmation sign becomes visible with no judgment line.



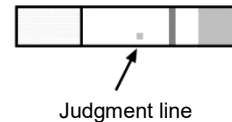
It is assumed that protein originating from food allergen is not contained in the test sample, or that it is lower than 2 ppm even if it is contained.

- Re-testing: The confirmation sign is not visible.

It is possible that the test was not conducted properly if the confirmation sign does not become visible. Hold the judgment and conduct the test again with a new test strip and detection reagent.

Cautionary notes in making judgments

- (1) To obtain accurate judgment results, judge the result 20 minutes after inserting the test strip into the tube, and not any later. The judgment line may become visible later than 20 minutes when a low concentration of protein below the detection sensitivity is contained in the test sample, due to nonspecific reactions and so forth.
- (2) In case of food products which have a high viscosity or a high degree of coloring, conduct the test after an appropriate dilution, as the accurate judgment may be impossible.
- (3) Hold the judgment and conduct a test again using a new test strip and detection reagent if only a part of the line becomes visible instead of the entire line as shown in the figure on the right.



Reactivity with food products

- (1) Refer to the “Food product reactivity list” and “False negative list” which are disclosed on our company’s website* for checking reactivity with food products.
- (2) A phenomenon in which the judgment line does not become visible (prozone phenomenon) may occur if the concentration of the protein originating from the food allergen is extremely high. Re-test by diluting the test sample to 10 times with the specimen extraction solution in this case.

* <https://biochemifa.kikkoman.co.jp/e/>.



Storage of the results

- (1) If it is necessary to store the results, it is possible to tape the test strip on a sheet of copy paper and so forth. In this case, it is recommended that the sample pad part at the end of the test strip is cut off with scissors or that the test sample is absorbed by pressing some tissue paper over it.
- (2) The test date, sample name, judgment result and so forth can be written in the part above the judgment paper of the test strip (handle part).
- (3) Judgment line may appear or its color depth may change in time. In addition, the judgment paper part may acquire yellowish color in “Allergeneye Immunochromatography, Peanut.”

Precautions for use and handling

1. General precautions

- (1) This kit is a qualitative reagent to detect the protein originating from food allergen contained in food products or swabbing solution. Do not use it for any other purpose.
- (2) Do not use any expired reagents (the expiration date is indicated on the outer box, transparent plastic bag and so forth).
- (3) When testing with this kit, do so at the room temperature while avoiding direct sunlight and other heat sources.
- (4) Follow the ordinances of the local municipality when disposing of this kit. The test strips (PET, etc.) and desiccant (pulp) can be disposed by incineration.
 - * Materials: Transparent plastic bag (PE), aluminum bag (PE, etc.), detection reagent tube (PP), specimen extraction solution container (main body: PE, lid: PP), label (PET), outer box/partitioning (paper), Instruction Manual (paper)

2. Precautions in operation

- (1) To avoid making judgments on allergen content difficult with contamination from the experiment tools, wash the experiment tools to be used with neutral detergent, then soak them in an alkaline detergent and so forth overnight, rinse them well with tap water and then with purified water.
- (2) Since it is heated in boiling water, use heat-resistant gloves and so forth in handling, and work with caution not to suffer a burning injury.
- (3) Be careful not to allow no-water heating with the equipment used for heating.
- (4) Wash your hands thoroughly before testing, as allergen on the hands may contaminate the sample even if it is in a trace amount.
- (5) Open the transparent plastic bag containing the test strips immediately before using after it is brought up to the room temperature. Do not use the test strip if the bag is damaged, as it may deliver a false result.
- (6) Do not touch the sample pad. It may deteriorate the absorption of the specimen.
- (7) Proper judgment will be impossible if the judgment paper is wetted before use. In this case, stop using the test strip and use a new test strip to test.
- (8) A phenomenon in which the judgment line does not become visible (prozone phenomenon) may occur if the concentration of the protein originating from the food allergen in sample is extremely high. If prozone phenomenon is suspected, re-test by diluting the test sample to 10 times with the specimen extraction solution.

3. Precautions for prevention of danger

- (1) Do not allow the reagents of this kit to adhere to the skin, mucous membrane, clothes and so forth.
- (2) "Extraction solution (2) (10-time concentrated)" contains sodium azide. Drain with a large amount of water when disposing of it.
- (3) If a reagent has come in contact with the eye or mouth by mistake, immediately take the first-aid measures to rinse thoroughly with tap water and so forth, then seek consultation of a doctor.

4. Others

- (1) Evaluate the judgment result with this kit comprehensively in combination with the manufacture records, results of other measurement methods and so forth.
- (2) We will not be held liable for any damages or losses occurring as a consequence of using the test results of this kit.
- (3) "Allergeneye" is a registered trademark of Prima Meat Packers, LTD. in Japan and other countries.

Warranty

Kikkoman Biochemifa Company warrants the products in this kit to have a certain level of quality. This warranty guarantees that Kikkoman Biochemifa Company shall replace defective products should any be found. This warranty does not provide any other guarantees. Kikkoman Biochemifa Company shall not be liable for any damages, including special or consequential damages, or expenses arising directly or indirectly from the use of this product.



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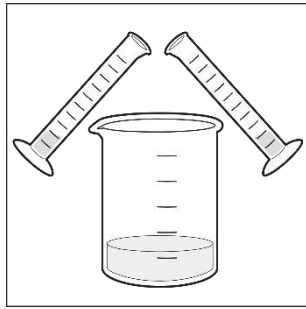
E-mail: biochemifa@mail.kikkoman.co.jp

URL: <https://biochemifa.kikkoman.co.jp/e/>

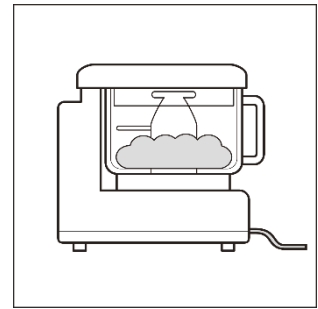
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Short instructions

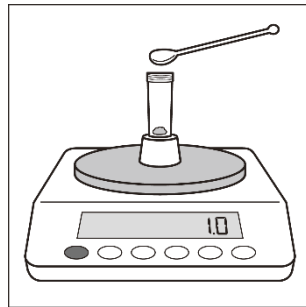
1. Prepare the specimen extraction solution. Add 10-time concentrated extraction solutions (1) and (2) to purified water and mix.



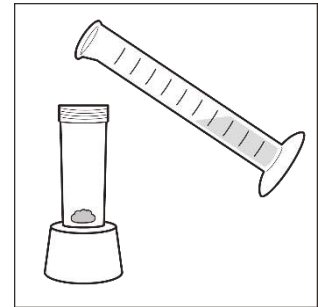
2. Homogenize the test sample.



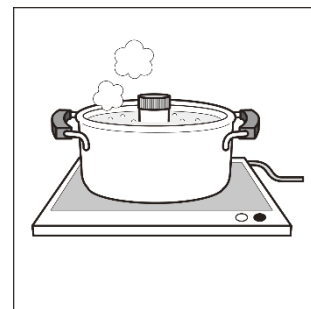
3. Measure 1 g of the test sample into a 50 mL centrifuge tube.



4. Add 19 mL specimen extraction solution and mix.



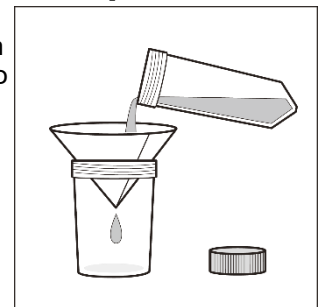
5. Heat it in a boiling water bath for 10 minutes. Then cool it to room temperature.



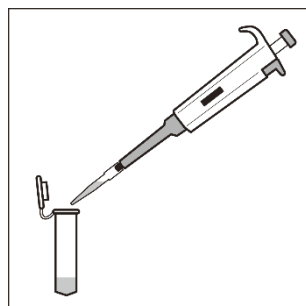
6. Filter the solution with a filter paper to obtain the test sample.

[Only when testing for wheat]

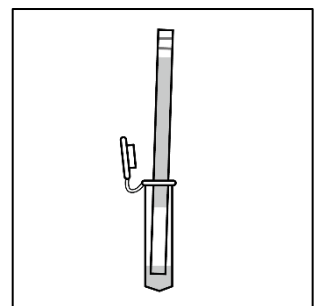
Dilute the filtrate 5 times with specimen extraction solution to use it as the test sample.



7. Add 100 μ L of the test sample to the detection reagent and shake to mix.



8. Insert a test strip and let it stand.



9. Pull out the test strip after 20 minutes to read the result.

