



- Rapid Test Pro II for Egg (Cat.# M2261)**
- Rapid Test Pro II for Total Milk (Cat.# M2269)**
- Rapid Test Pro II for Gluten (Cat.# M2263)**
- Rapid Test Pro II for Buckwheat (Cat.# M2264)**
- Rapid Test Pro II for Peanut (Cat.# M2265)**
- Rapid Test Pro II for Crustacean (Cat.# M2267)**
- Rapid Test Pro II for Walnut (Cat.# M2268)**
- Rapid Test Pro II for Soya (Cat.# M2266)**
- Rapid Test Pro II for Shellfish (Cat.# M2270)**

For the Quick Detection of Protein of Allergenic Ingredients
in Foods and on Food-processing Equipment

10 tests

For Research or Laboratory Use Only

Not for Use in Diagnostic Procedures

Please read full descriptions in this manual before use

Manufactured by:

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Warnings

- Do not combine reagents from different lots.
- Store the kit at 2–8°C (35–46°F), and DO NOT FREEZE.
- Do not use the kit after the expiration date indicated on the box.

1. Intended Use

Rapid Test Pro II is intended for the quick detection of protein from allergenic ingredients in unprocessed samples, processed food, on environmental surfaces (swab test) and in rinse water.

NOTE: For the analysis of environmental surfaces (swab test) and rinse water, we recommend using the Rapid Test Easy kits.

2. Description of the Product

- A qualitative test in lateral flow immunoassay format for visual detection
- Provides test results in 15 minutes (Including sample preparation: within 30 minutes)
- Improved recovery of protein from both unprocessed and processed food products by using the patented extraction solution¹⁻⁴⁾
- Rapid Test Pro II for Total Milk has two test lines on a test stick to detect casein and β -lactoglobulin respectively.
- Performance characteristics of each kit are shown in Tables 1-9.

Table 1. Performance characteristics of Rapid Test Pro II for Egg

Limit of detection	Food:	5 μ g/g (5 ppm) Egg protein
	Surfaces (swab test):	1 μ g Egg protein/100 cm ²
	Rinse water:	5 μ g/mL (5 ppm) Egg protein
Specificity	The antibody reacts with Ovalbumin.	

Table 2. Performance characteristics of Rapid Test Pro II for Total Milk

Line 1 (black, upstream side)	Limit of detection	Food: 4 μ g/g (4 ppm) Casein Surfaces (swab test): 0.8 μ g Casein/100 cm ² Rinse water: 4 μ g/mL (4 ppm) Casein (4 μ g/g Casein correspond to 5 μ g/g (5 ppm) milk protein)
	Specificity	The antibody reacts with Casein.
Line 2 (red, downstream side)	Limit of detection	Food: 0.5 μ g/g (0.5 ppm) β -lactoglobulin Surfaces (swab test): 0.1 μ g β -lactoglobulin/100 cm ² Rinse water: 0.5 μ g/mL (0.5 ppm) β -lactoglobulin (0.5 μ g/g β -lactoglobulin correspond to 5 μ g/g (5 ppm) milk protein)
	Specificity	The antibody reacts with β -lactoglobulin.

Table 3. Performance characteristics of Rapid Test Pro II for Gluten

Limit of detection	Food:	4 μ g/g (4 ppm) Gluten
	Surfaces (swab test):	0.8 μ g Gluten/100 cm ²
	Rinse water:	4 μ g/mL (4 ppm) Gluten protein (4 μ g/g Gluten correspond to 5 μ g/g (5 ppm) wheat protein)
Specificity	The antibody reacts with Gliadin*.	

*Cross reacts to barley and rye

Table 4. Performance characteristics of Rapid Test Pro II for Buckwheat

Limit of detection	Food:	5 μ g/g (5 ppm) Buckwheat protein
	Surfaces (swab test):	1 μ g Buckwheat protein/100 cm ²
	Rinse water:	5 μ g/mL (5 ppm) Buckwheat protein
Specificity	The antibody reacts with multiple buckwheat proteins.	

Table 5. Performance characteristics of Rapid Test Pro II for Peanut

Limit of detection	Food:	5 μ g/g (5 ppm) Peanut protein
	Surfaces (swab test):	1 μ g Peanut protein/100 cm ²
	Rinse water:	5 μ g/mL (5 ppm) Peanut protein
Specificity	The antibody reacts with multiple peanut proteins.	

Table 6. Performance characteristics of Rapid Test Pro II for Crustacean

Limit of detection	Food:	5 μ g/g (5 ppm) Crustacean protein
	Surfaces (swab test):	1 μ g Crustacean protein/100 cm ²
	Rinse water:	5 μ g/mL (5 ppm) Crustacean protein
Specificity	The antibody reacts with Crustacean Tropomyosin.	

Table 7. Performance characteristics of Rapid Test Pro II for Walnut

Limit of detection	Food:	5 μ g/g (5 ppm) Walnut protein
	Surfaces (swab test):	1 μ g Walnut protein/100 cm ²
	Rinse water:	5 μ g/mL (5 ppm) Walnut protein
Specificity	The antibody reacts with 2S albumin protein.	

Table 8. Performance characteristics of Rapid Test Pro II for Soya

Limit of detection	Food:	5 μ g/g (5 ppm) Soya protein
	Surfaces (swab test):	1 μ g Soya protein/100 cm ²
	Rinse water:	5 μ g/mL (5 ppm) Soya protein
Specificity	The antibody reacts with β -conglycinin.	

Table 9. Performance characteristics of Rapid Test Pro II for Shellfish

Limit of detection	Food:	5 μ g/g (5 ppm) Shellfish protein
	Surfaces (swab test):	1 μ g Shellfish protein/100 cm ²
	Rinse water:	5 μ g/mL (5 ppm) Shellfish protein
Specificity	The antibody reacts with Shellfish Tropomyosin.	
Reactivity	This kit detects shrimp, crab, lobster, squid, octopus and oyster proteins.	

3. Kit Components

Component	Amount
Extraction Solution*	10 packs (19 mL/pack)
Diluent	1 bottle (12 mL)
Test Stick	10 packs (1 stick/pack)
Pipette (L)	10
Pipette (S)	10
Polypropylene tube (L), 50 mL volume	10
Polypropylene tube (S), 1.5 mL volume	10
Cotton swab	10 packs
Paper tube rack	1

* **Extraction Solutions** for all test kits are identical. **Extraction Solution** may contain precipitates when refrigerated which should dissolve upon warming to 30–37°C (86–99°F).

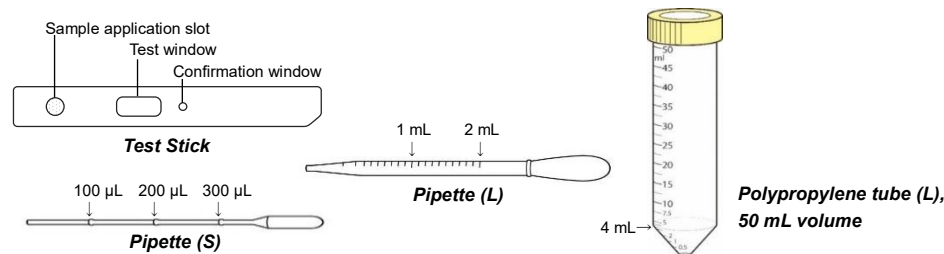


Fig. 1. Components (Test Stick, Pipette (L), Pipette (S) and Polypropylene tube (L))

4. Materials required but not provided

Precautions

- ✓ All procedures should be performed under contamination-free conditions to obtain reliable results. Make sure to avoid cross-contaminations via equipment, devices, tubes, containers, pipette tips, etc. The use of disposable materials is recommended.

- Homogenizer/blender
- Scale capable of weighing 1.0±0.1 g
- Vortex mixer
- Water bath*
- Centrifuge (for 3000 x g)*
- Filter paper*
- pH test strip
- Heat-resistant glove

*The items may not be required depending on the extraction method or sample condition.

5. Sample Extraction

Precautions

- ✓ Prior to use, bring all reagents to 20–30°C (68–86°F) and gently vortex the contents into a homogeneous solution.
- ✓ Confirm and adjust the pH of **Sample Extract** close to neutral (pH 6–8) as required.
- ✓ Wear suitable protective clothing, goggles and gloves when handling the kit.

[A. Recommended Extraction Method]

This extraction method is available for all food, environmental surface (swab test) and in rinse water.

In particular, this is optimal for highly processed food. Please choose this method for the analysis of baked goods (bread, confectionery), retort foods, processed meat/seafood products, stewing foods.

A-1. For food sample

- Grind and mix the test food sample to homogeneity with a contamination-free homogenizer/blender.
 - Put 1.0 g (1.0 mL) of the homogenized sample in a **Polypropylene tube (L)** and add 1 pack of **Extraction Solution**.
 - Close the tube tightly and vortex it for 30 seconds.
 - Place the closed tube in a water bath > 90°C (194°F) for 10 minutes.
 - Place the tube in water to cool down to ambient temperature.
- NOTE:** Do not cool down below ambient as the sample precipitates at low temperatures.
- Vortex for 30 seconds.
 - Place the tube in a stand for a few minutes to let the sample settle down, and then collect the supernatant as **Sample Extract**.
- NOTE:** Centrifuge and/or filter with filter paper as required.

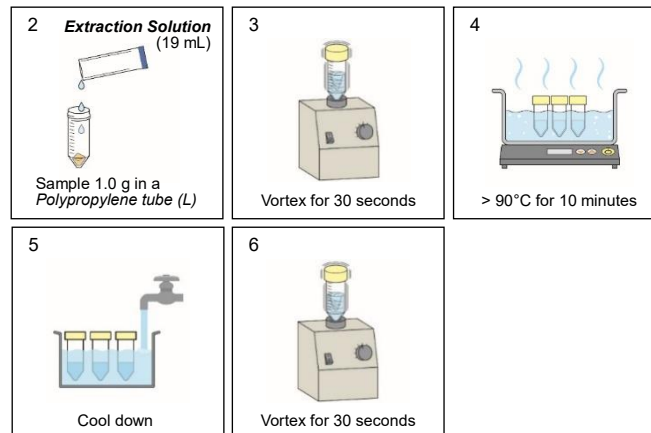


Fig. 2. Sample extraction for food sample

A-2. For swab test sample

- Thoroughly wipe across (zigzagging) the specified surface area of 10 cm × 10 cm with a **Cotton swab** moistened with purified water 1st pass in diagonal, and a 2nd pass in diagonal perpendicular to the 1st pass.
 - Place the swab into a **Polypropylene tube (L)** and add 4 mL of **Extraction Solution** (Sensitivity: 1 μ g (0.8 μ g for Casein and Gluten, 0.1 μ g for β -lactoglobulin) on the swab in the case of adding 4 mL, see Tables 1-8).
 - Close the tube tightly and vortex it for 30 seconds.
 - Place the closed tube in a water bath > 90°C (194°F) for 10 minutes.
 - Place the tube in water to cool down to ambient temperature.
- NOTE:** Do not cool down below ambient as the sample precipitates at low temperatures.
- Vortex for 30 seconds. The resulting solution is referred to as **Sample Extract**.
- NOTE:** Filter it with filter paper as required.

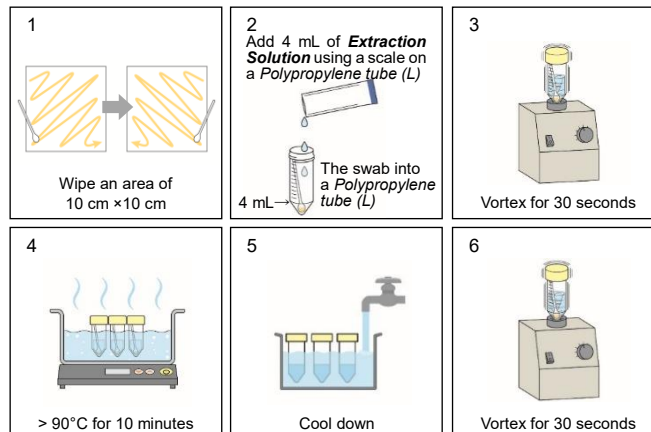


Fig. 3. Sample extraction for swab test sample

A-3. For rinse water sample

- 1. Put 1.0 mL of the sample in a *Polypropylene tube (L)* and add 1 pack of **Extraction Solution**.
- 2. Close the tube tightly and vortex it for 30 seconds.
- 3. Place the closed tube in a water bath > 90°C (194°F) for 10 minutes.
- 4. Place the tube in water to cool down to ambient temperature.
- NOTE:** Do not cool down below ambient as the sample precipitates at low temperatures.
- 5. Vortex for 30 seconds. The resulting solution is referred to as **Sample Extract**.

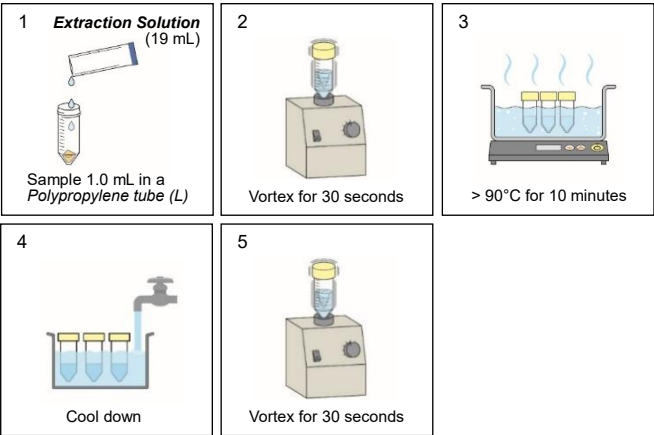


Fig. 4. Sample extraction for rinse water sample

【B. Simplified Extraction Method】

This extraction method is available for food which is not highly processed, environmental surface (swab) tests and rinse water. Please choose this method for the analysis of beverage, ice cream, dairy products, dessert, grain powder, premix and other not heat-treated products. For further information please contact us when you need.

B-1. For food sample

- 1. Grind and mix the test food sample to homogeneity with a contamination-free homogenizer/blender.
- 2. Put 1.0 g (1.0 mL) of the homogenized sample in a *Polypropylene tube (L)* and add 1 pack of **Extraction Solution**.
- 3. Close the tube tightly and vortex it for 30 seconds.
- NOTE:** Centrifuge and/or filter with filter paper as required.

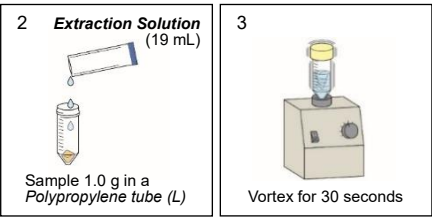


Fig. 5. Sample extraction for food sample

B-2. For swab test sample

- 1. Thoroughly wipe across (zigzagging) the specified surface area of 10 cm × 10 cm with a *Cotton swab* moistened with purified water 1st pass in diagonal, and a 2nd pass in diagonal perpendicular to the 1st pass.
- 2. Place the swab into a *Polypropylene tube (L)* and add 4 mL of **Extraction Solution** (Sensitivity: 1 µg (0.8 µg for Casein and Gluten, 0.1 µg for β-lactoglobulin) on the swab in the case of adding 4 mL, see Tables 1-8).
- 3. Close the tube tightly and vortex it for 30 seconds.
- NOTE:** Filter it with filter paper as required.

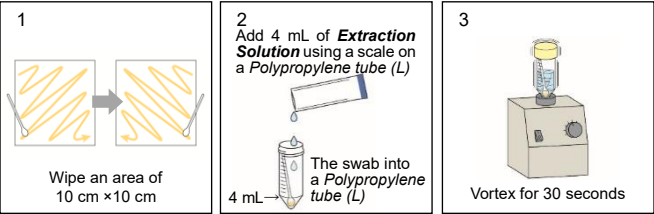


Fig. 6. Sample extraction for swab test sample

B-3. For rinse water sample

- 1. Put 1.0 mL of the sample in a *Polypropylene tube (L)* and add 1 pack of **Extraction Solution**.
- 2. Close the tube tightly and vortex it for 30 seconds.

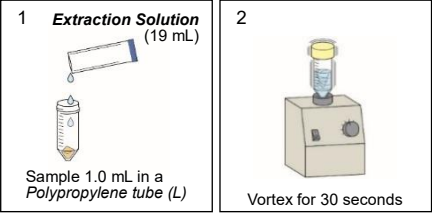


Fig. 7. Sample extraction for rinse water sample

6. Preparation of Test Solution

- 1. Dispense 900 µL of **Diluent** with a *Pipette (L)* into a *Polypropylene tube (S)*.
- 2. Add 100 µL of **Sample Extract** with a *Pipette (S)* to the *Polypropylene tube (S)* containing 900 µL of **Diluent** and mix well. The resulting solution is referred to as **Test Solution**.
- NOTE:** For further dilution, dilute the **Sample Extract** with **Extraction Solution** appropriately, and then dilute it 10-fold with **Diluent**.

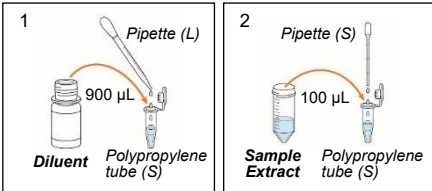


Fig. 8. Preparation of Test Solution

7. Test Procedures

Precautions

- ✓ Prior to use, adjust the temperature of a **Test Stick** to 20–30°C (68–86°F) and open the package just before use. At low-temperature the **Test Stick** may not work properly.
- ✓ Neither touch the sample application slot nor the test window of a **Test Stick**.

- 1. Place a **Test Stick** horizontally and add 200 µL of **Test Solution** to the sample application slot.
- 2. Incubate 15 minutes at room temperature (20-30°C/ 68-86°F) in a flat and horizontal surface.
- 3. Immediately interpret the results according to **8. Results** described below.

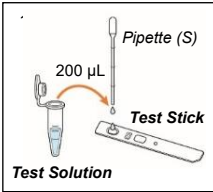


Fig. 9. Test step

8. Results

【A. Rapid Test ProII for Egg, Gluten, Buckwheat, Peanut, Crustacean, Walnut , Soya and Shellfish】

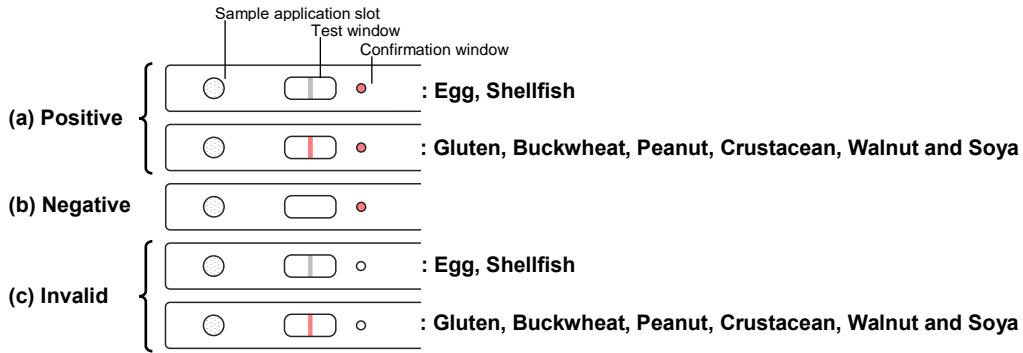


Fig. 10. Interpretation of results (Rapid Test ProII for Egg, Gluten, Buckwheat, Peanut, Crustacean, Walnut, Soya and Shellfish)

- (a) Positive: A black or red line in a test window together with red color in a confirmation window.
- (b) Negative: No line in a test window together with a red color in a confirmation window.
- (c) Invalid: No color in a confirmation window.

【B. Rapid Test ProII for Total Milk】

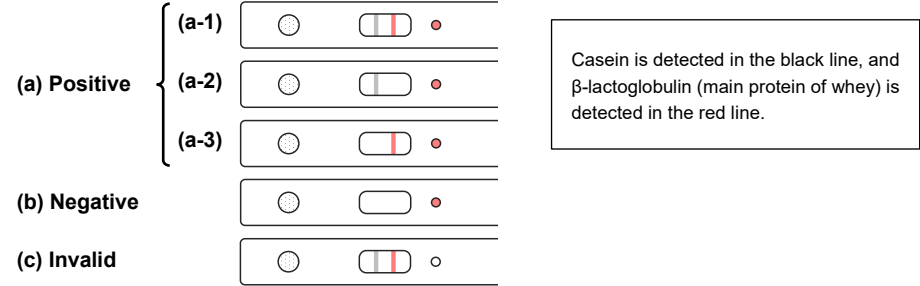


Fig. 11. Interpretation of results (Rapid Test ProII for Total Milk)

- (a) Positive: (a-1) Two lines, a black and a red line, in a test window together with red color in a confirmation window (when milk component is contained). (a-2) A black line in a test window together with red color in a confirmation window (when only casein is contained*). (a-3) A red line in a test window together with red color in a confirmation window (when only whey is contained*).
- (b) Negative: No line in a test window together with a red color in a confirmation window.
- (c) Invalid: No color in a confirmation window.

* Since casein, which is commonly available on the market, contains trace amounts of β-lactoglobulin in addition to casein, and whey contains trace amounts of casein in addition to β-lactoglobulin, two lines may also be observed even when testing foods containing only casein or whey.

NOTE: If there is no color in a confirmation window, retest with a new **Test Stick**.

False-negative results may occur depending on the condition of the target protein (e.g. degradation).

If false-negative results occur at high concentrations of the target protein (hook effect), retest with a diluted **Test Solution** (see **6. Preparation of Test Solution**).

9. References

- 1. Patent No.: JP 5133663,
- 2. Patent No.: AU 2008330507,
- 3. Patent No.: US 8,859,212,
- 4. Patent No.: EP 2224239 (AT, BE, DE, ES, FR, GB, IT, NL, CH)

10. Warranties

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11. Appendix: Test Flow Chart

	Food sample Rinse water sample	Swab test sample
5. Sample Extraction	Mix and homogenize a sample (for Food sample)	Swab a specified area
	▼	▼
	Put 1.0 g (mL) of the homogenized sample in a tube and add 1 pack of Extraction Solution	Place the swab into the tube and add 4 mL of Extraction Solution
	▼	▼
	Vortex for 30 seconds	
	▼	▼
	Place the tube in a water bath > 90° C (194° F) for 10 minutes and cool down	
6. Preparation of Test Solution	▼	▼
	Vortex for 30 seconds	
	▼	▼
7. Test Procedures	Let the sample settle down and collect the supernatant as Sample Extract	
	▼	▼
8. Results	Add 100 µL of Sample Extract to 900 µL of Diluent and mix well as Test Solution	
	▼	▼
8. Results	Add 200 µL of Test Solution to a Test Stick	
	▼	▼
8. Results	Check results in 15 minutes	