

Rapid Test Pro II for

Egg	(Cat.# M2261)	Total Milk	(Cat.# M2269)
Gluten	(Cat.# M2263)	Buckwheat	(Cat.# M2264)
Peanut	(Cat.# M2265)	Crustacean	(Cat.# M2267)
Walnut	(Cat.# M2268)	Cashew	(Cat.# M2260)
Soya	(Cat.# M2266)	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

1. Do not combine reagents from different lots.
2. Store the kit at 2–8°C (35–46°F), and DO NOT FREEZE.
3. 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.

2. Description of the Product

- A qualitative test in lateral flow immunoassay format for visual detection
- Provides test results in 15 or 30 minutes (Including sample preparation: within 45 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, 2.

Table 1. Performance characteristics for 15-minute method

Kit (Cat.#)		Analyte	Limit of Detection			
			Food	Surfaces (swab test)	Rinse water	Reporting unit
Egg (M2261)		Ovalbumin	5 µg/g (5 ppm)	1 µg/100 cm ²	5 µg/mL (5 ppm)	Whole egg protein
Total Milk (M2269)	Line1	Casein	4 µg/g (4 ppm)	0.8 µg/100 cm ²	4 µg/mL (4 ppm)	Casein
	Line2	Beta- Lactoglobulin	0.5 µg/g (0.5 ppm)	0.1 µg/100 cm ²	0.5 µg/mL (0.5 ppm)	Beta- Lactoglobulin
Gluten (M2263)		Gliadin	4 µg/g (4 ppm)	0.8 µg/100 cm ²	4 µg/mL (4 ppm)	Gluten
Buckwheat (M2264)		Partially purified buckwheat proteins	5 µg/g (5 ppm)	1 µg/100 cm ²	5 µg/mL (5 ppm)	Whole Buckwheat protein
Peanut (M2265)		Partially purified peanut proteins				Whole Peanut protein
Crustacean (M2267)		Crustacean tropomyosin				Whole Crustacean protein
Walnut (M2268)		Walnut 2S albumin				Whole Walnut protein
Cashew (M2260)		Cashew 2S albumin				Whole Cashew protein
Soya (M2266)		β-conglycinin				Whole Soya protein
Shellfish (M2270)		Shellfish tropomyosin				Whole Shellfish protein

Table 2. Performance characteristics for 30-minute method

Kit (Cat.#)	Analyte	Limit of Detection			
		Food	Surfaces (swab test)	Rinse water	Reporting unit
Egg (M2261)	Ovalbumin	1 µg/g (1 ppm)	0.2 µg/100 cm ²	1 µg/mL (1 ppm)	Whole egg protein
Total Milk (M2269)	Line1 Casein	0.8 µg/g (0.8 ppm)	0.16 µg/100 cm ²	0.8 µg/mL (0.8 ppm)	Casein
	Line2 Beta- Lactoglobulin	0.1 µg/g (0.1 ppm)	0.02 µg/100 cm ²	0.1 µg/mL (0.1 ppm)	Beta- Lactoglobulin
Gluten (M2263)	Gliadin	0.8 µg/g (0.8 ppm)	0.16 µg/100 cm ²	0.1 µg/mL (0.1 ppm)	Gluten

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).

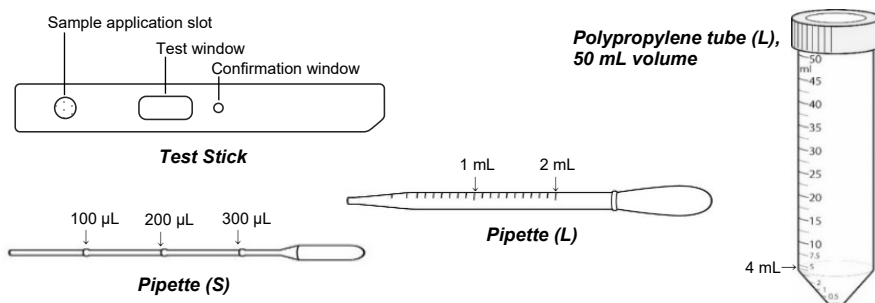


Fig. 1. Components (Test Stick, Pipette (L), Pipette (S), and Polypropylene tube (L))

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, etc. The use of disposable materials is recommended.

-
- Vortex mixer
 - Timer
 - Homogenizer/blender*
 - Scale capable of weighing 1 g*
 - Water bath*
 - Centrifuge (for 3,000 x g) *
 - Filter paper*
 - pH test strip*
 - Heat-resistant glove*
 - Protective clothing, goggles, and gloves*

* 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.

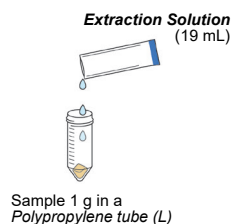
【A. Thermal Extraction Method】

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

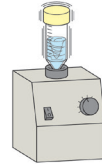
In particular, this is optimal for highly processed food. Please choose this method for the analysis of baked goods (bread, confectionery), retort foods, processed meat/seafood products, stewing foods.

A-1. For food sample

1. Grind and mix the test food sample to homogeneity with a contamination-free homogenizer/blender.
2. Put 1 g (1 mL) of the homogenized sample in a *Polypropylene tube (L)* and add 1 pack of **Extraction Solution**.

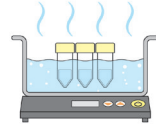


3. Close the tube tightly and vortex it for 30 seconds.

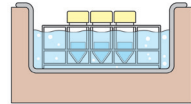


Vortex for 30 seconds

4. Place the closed tube in a water bath $> 90^{\circ}\text{C}$ (194°F) for 10 minutes.

 $> 90^{\circ}\text{C}$ for 10 minutes

5. Place the tube in water to cool down to ambient temperature.
Do not cool down below ambient as the sample precipitates at low temperatures.



Cool down

6. Vortex for 30 seconds and place the tube in a stand for a few minutes to let the sample settle down, and then collect the supernatant as **Sample Extract**.
Centrifuge and/or filter with filter paper as required.



Vortex for 30 seconds

A-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.



Wipe an area of
10 cm × 10 cm

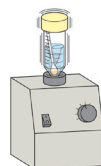
2. Place the swab into a *Polypropylene tube (L)* and add 4 mL of **Extraction Solution** (Sensitivity: 1 µg (0.8 µg for Casein and Gluten, 0.1 µg for β-lactoglobulin) on the swab in the case of adding 4 mL, see Tables 1, 2).

Add 4 mL of **Extraction Solution** using a scale on a *Polypropylene tube (L)*



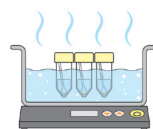
The swab into a *Polypropylene tube (L)*
4 mL →

3. Close the tube tightly and vortex it for 30 seconds.



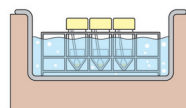
Vortex for 30 seconds

4. Place the closed tube in a water bath > 90°C (194°F) for 10 minutes.



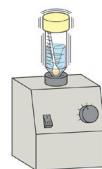
> 90°C for 10 minutes

5. Place the tube in water to cool down to ambient temperature.
Do not cool down below ambient as the sample precipitates at low temperatures.



Cool down

6. Vortex for 30 seconds. The resulting solution is referred to as **Sample Extract**.
Filter it with filter paper as required.



Vortex for 30 seconds

A-3. For rinse water sample

1. Put 1 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 $> 90^{\circ}\text{C}$ (194°F) for 10 minutes.
4. Place the tube in water to cool down to ambient temperature.
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**.

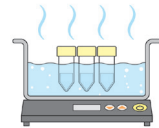
Extraction Solution
(19 mL)



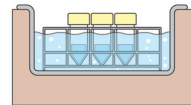
Sample 1 mL in a
Polypropylene tube (L)



Vortex for 30 seconds



$> 90^{\circ}\text{C}$ for 10 minutes



Cool down



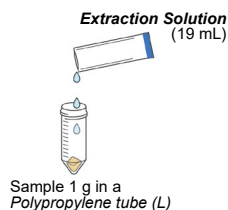
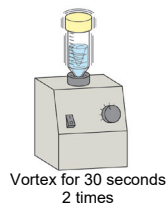
Vortex for 30 seconds

【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 g (1 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 2 times.
Place the tube in a stand for a few minutes to let the sample settle down, and then collect the supernatant as **Sample Extract**. Centrifuge and/or filter with filter paper as required.



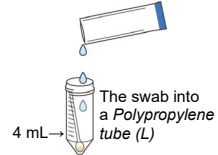
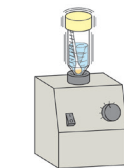
B-2. For swab test sample

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, 2).
3. Close the tube tightly and vortex it for 30 seconds 2 times.
The resulting solution is referred to as **Sample Extract**.
Filter it with filter paper as required.



Wipe an area of
10 cm × 10 cm

Add 4 mL of **Extraction Solution** using a scale on a *Polypropylene tube (L)*



Vortex for 30 seconds
2 times

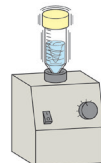
B-3. For rinse water sample

1. Put 1 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 2 times.
The resulting solution is referred to as **Sample Extract**.

Extraction Solution
(19 mL)



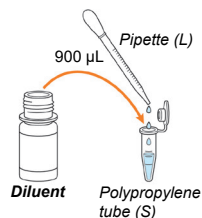
Sample 1 mL in a
Polypropylene tube (L)



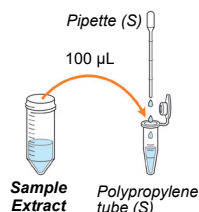
Vortex for 30 seconds
2 times

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**.

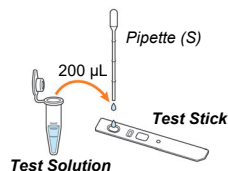
7. Test Procedures for 15-minute method

Applicable Products: All of Rapid Test Pro II

✓ 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 **9. Results** described below.



8. Test Procedures for 30-minute method

Applicable Products: Rapid Test Pro II for Egg, Total Milk and Gluten

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 **30 minutes** at room temperature (20–30°C/ 68–86°F) in a flat and horizontal surface.
3. Immediately interpret the results according to **9. Results** described below.

9. Results

[A. Rapid Test Pro II for Egg, Gluten, Buckwheat, Peanut, Crustacean, Walnut, Cashew, Soya, and Shellfish]

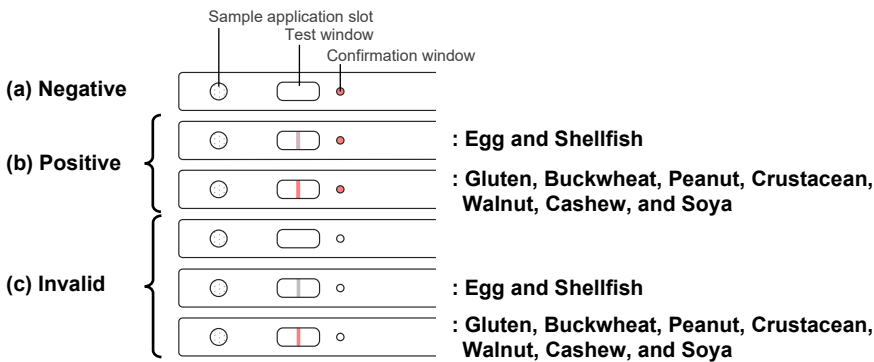


Fig. 2. Interpretation of results

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

【B. Rapid Test Pro II for Total Milk】



Fig. 3. Interpretation of results

- (a) Negative: No line in a test window together with a red color in a confirmation window.
- (b) Positive: (b-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).
 (b-2) A black line in a test window together with red color in a confirmation window (when only casein is contained).
 (b-3) A red line in a test window together with red color in a confirmation window (when only whey is contained).
- (c) Invalid: No color in a confirmation window.

NOTE

- If no color appears in the confirmation window, the test is invalid. Retest the sample using a new **Test Stick**.
- If the line is only partially visible in the test window, the result should be considered invalid. Retest using a new **Test Stick** and a freshly prepared Test Solution.

- 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**).
- For food reactivity data, please refer to our website.
<https://www.morinaga-biosci.com/product/index.html#link02>

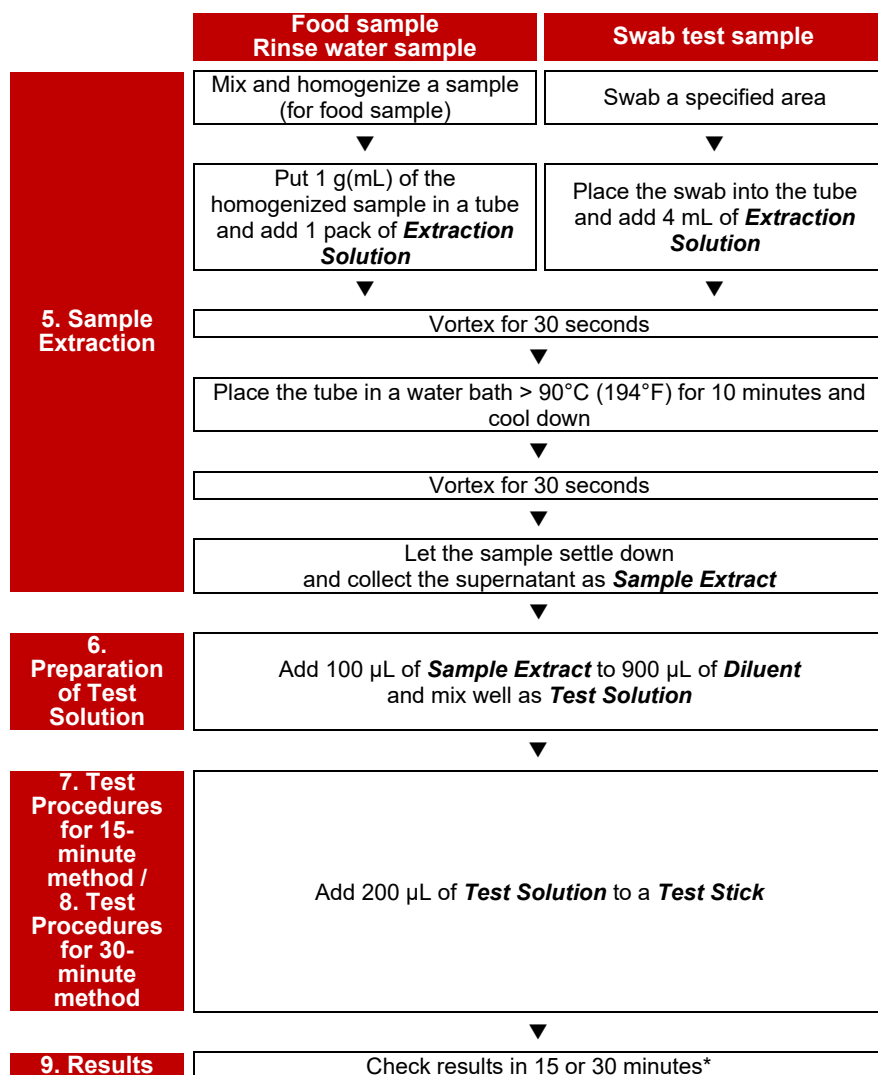
10. Reference

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)

11. Warranties

Morinaga BioScience, Inc. makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. Buyer assumes all risk and liability resulting from the use of this product. There is no warranty of merchantability of the product, or of the fitness of the product for any purpose. Morinaga BioScience, Inc. agrees to replace any defective product, but expressly disclaims liability for damages, including special or consequential damage, or expenses arising directly or indirectly from the use of this product.

12. Appendix: Test Flow Chart



*The 30-minute method is applicable to Rapid Test Pro II for Egg, Total Milk and Gluten.