V.5/Mar/2021

MORINAGA

Rapid Test Pro Ⅱ for Egg (Cat.# M2261)
Rapid Test Pro Ⅱ for Casein (Cat.# M2262)
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 Soya (Cat.# M2266)

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.

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 proteins from both unprocessed and processed food products by using the patented extraction solution[1-4)]
- Performance characteristics of each kit is shown in Tables 1-6

Table1. Performance characteristics of Rapid Test Pro Ⅱ for Egg
(Recommended and Simplified Extraction method)

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

Table2. Performance characteristics of Rapid Test Pro Ⅱ for Casein
(Recommended and Simplified Extraction method)

Limit of detection	Food: 4 µg/g (4 ppm) Casein Surfaces (swab test): 0.8 µg Casein / 100 cm^2 Rinse water: 4 µg/mL (4 ppm) Casein 4 µg/g Casein correspond to 5 µg/g (5 ppm) milk protein
Specificity	The polyclonal antibody reacts with Casein

Table3. Performance characteristics of Rapid Test Pro Ⅱ for Gluten
(Recommended and Simplified Extraction method)

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

*Cross reacts to barley and rye

Table4. Performance characteristics of Rapid Test Pro Ⅱ for Buckwheat
(Recommended and Simplified Extraction method)

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

Table5. Performance characteristics of Rapid Test Pro Ⅱ for Peanut
(Recommended and Simplified Extraction method)

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

Table6. Performance characteristics of Rapid Test Pro Ⅱ for Soya
(Recommended and Simplified Extraction method)

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

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).

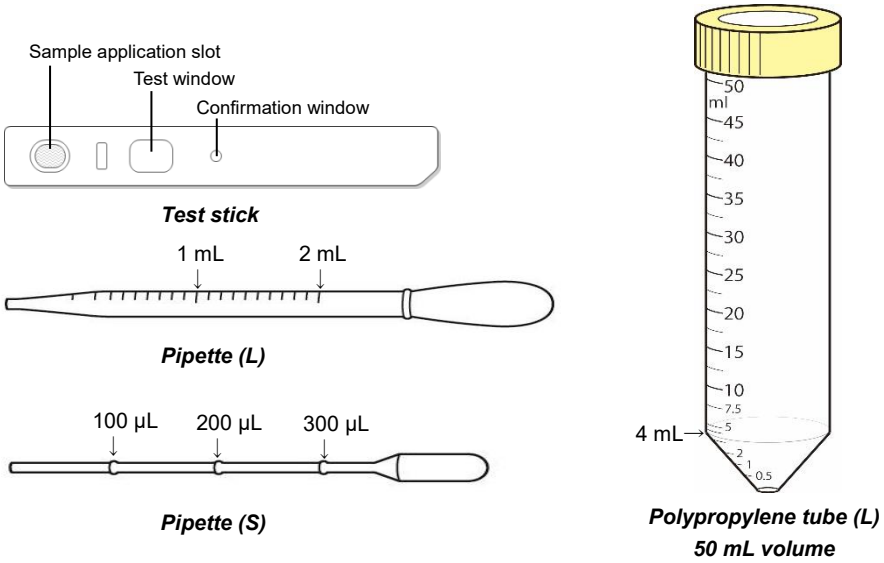

Fig1. Components (*Test stick, Pipette (L), Pipette (S) and Polypropylene tube (L)*)

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 test 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

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.
4. Place the closed tube in a water bath > 90°C (194˚F) for 10 minutes.
5. 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.
6. Vortex for 30 seconds.
7. 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.

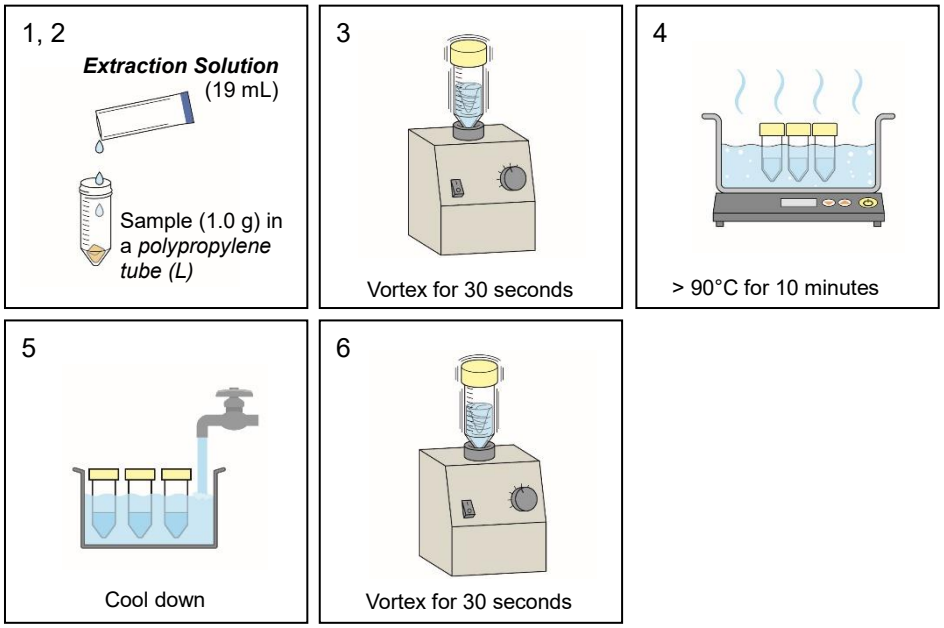

Fig.2 Sample extraction for food sample

A-2. For swab test sample

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 first pass.
2. Place the swab into a *polypropylene tube (L)* and add 4 mL of ***Extraction Solution***.
3. Close the tube tightly and vortex it for 30 seconds.
4. Place the closed tube in a water bath > 90°C (194˚F) for 10 minutes.
5. 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.
6. Vortex for 30 seconds. The resulting solution is referred to as ***Sample Extract***.
   NOTE: Filter it with filter paper as required.

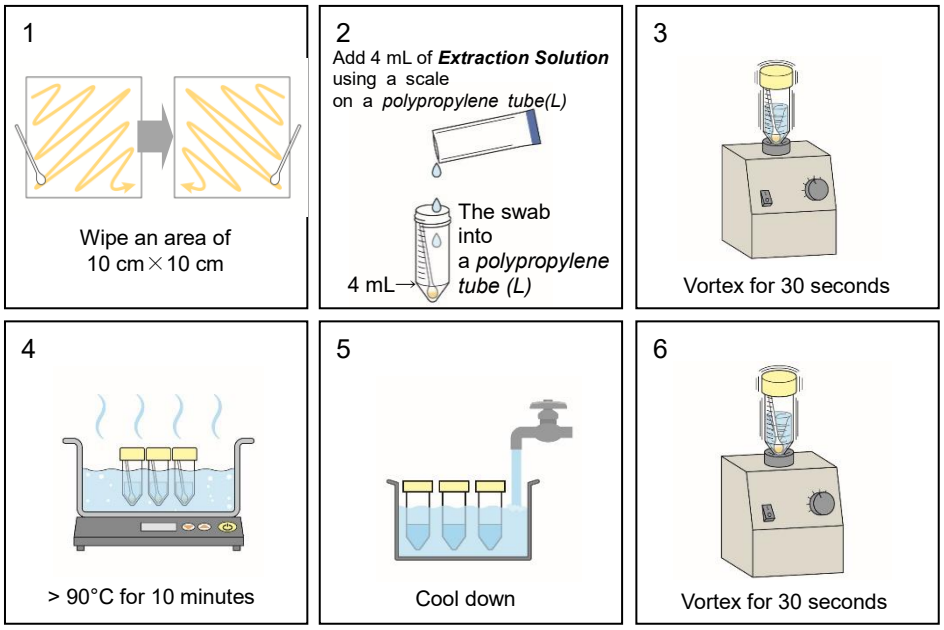

Fig.3 Sample extraction for swab test sample

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***.

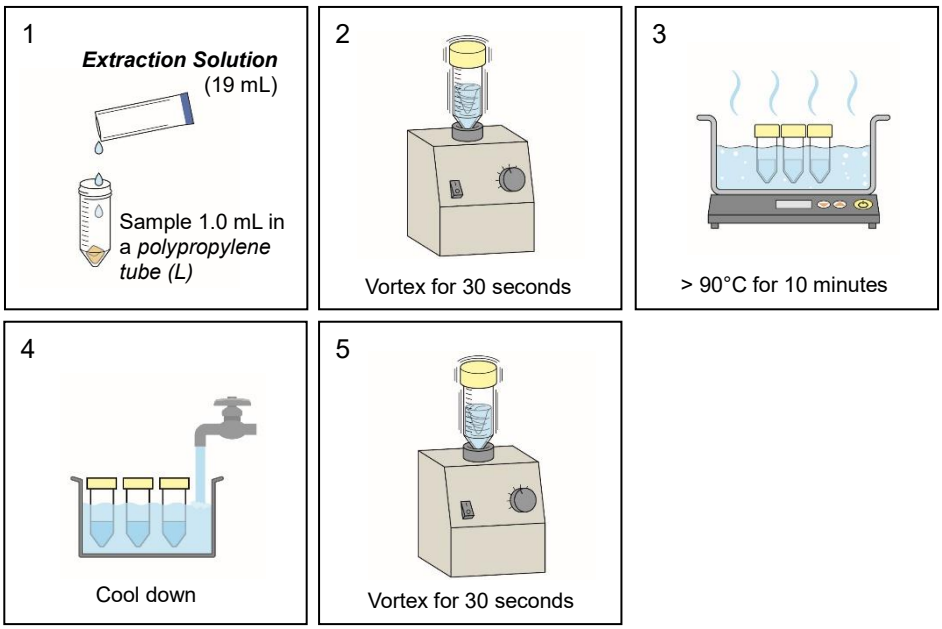

Fig. 4 Sample extraction for rinse water sample

【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. Vortex the capped tube for 30 seconds.
   NOTE: Centrifuge and/or filter with filter paper as required.

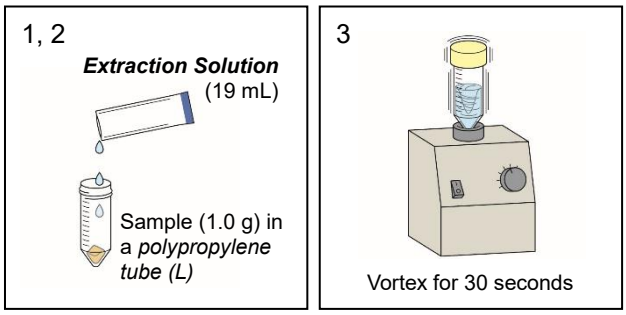

Fig.5 Sample extraction for food sample

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 first 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) on the swab in the case of adding 4 mL, *see* Table1-6)
3. Cap the tube tightly and vortex it for 30 seconds.
   NOTE: Filter it with filter paper as required.

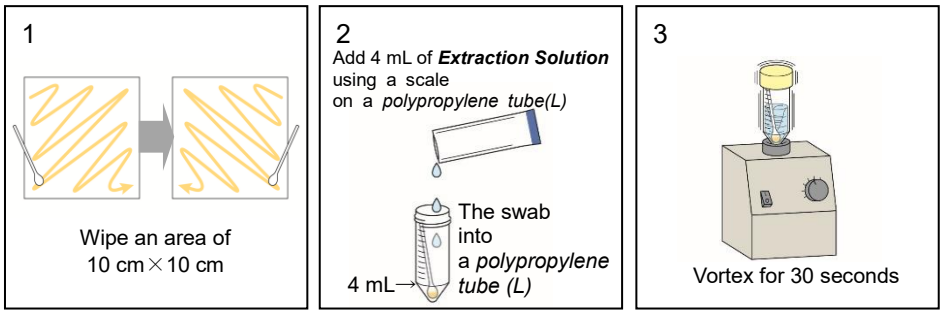

Fig.6 Sample extraction for swab test sample

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. Vortex the capped tube for 30 seconds.

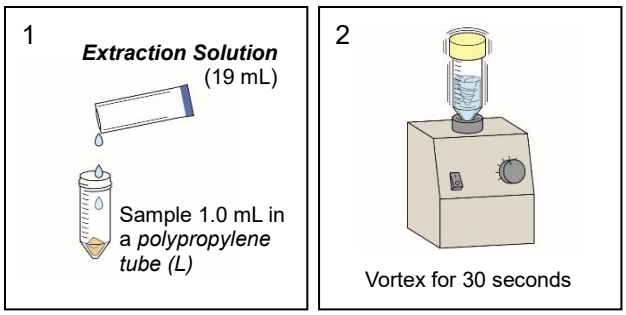

Fig.7 Sample extraction for rinse water sample

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***.

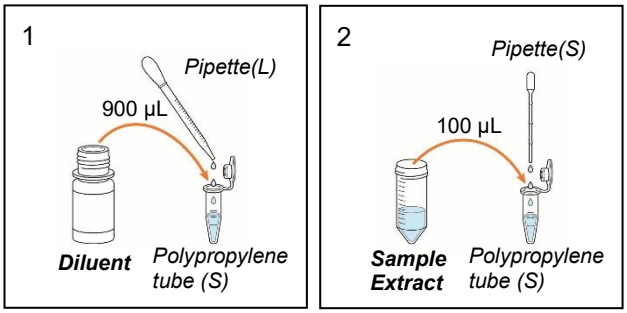

Fig. 8 Preparation of *Test Solution*

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.
NOTE: 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 in a flat and horizontal surface.
3. Immediately interpret the results according to ***8. Results*** described below.

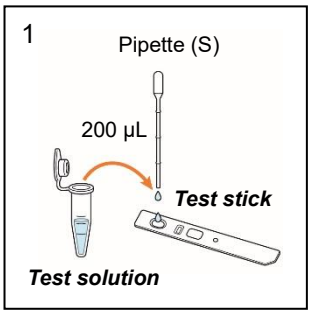

Fig.9 Test step

8. Results

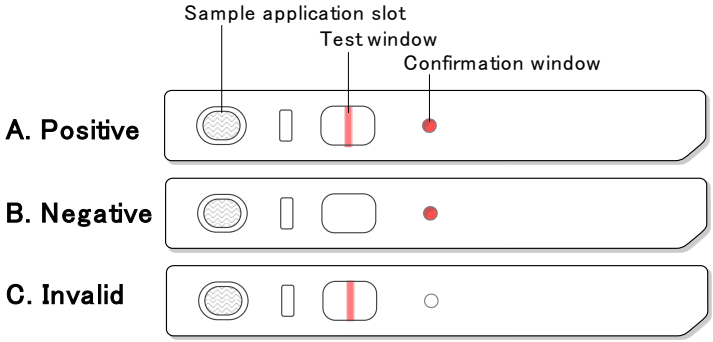

Fig10. Interpretation of results

A) Positive: A red-purple 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.

NOTE: If there is no color in a confirmation window, retest with a new stick. Negative results will occur if ***Test Solution*** contains protein less than detectable levels. False-negative results may occur depending on the condition of target protein (e.g. degradation). If false-negative results occur at high concentrations of 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

Morinaga Institute of Biological Science, 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 Institute of Biological Science, 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.

11. Appendix: Test Flow Chart