Cat. # 9192

For Research Use

TaKaRa

Fruit-mate™ for RNA Purification

Product Manual

v201907Da

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I. Description

Fruit-mate for RNA Purification (hereafter described as Fruit-mate) is a supportive reagent for RNA extraction by using RNAiso Plus or NucleoSpin RNA Plant. This product applies when extracting total RNA from plants sample (root crop, fruit, seed, etc.) that contains large quantities of polysaccharides or polyphenols. The Fruit-mate contains non-ionic polymer and binds to substances such as polysaccharides or polyphenols. These substances can be easily removed by adding this product to the homogenized sample, binding with the product, and performing centrifugation. The RNA extraction from plants which have been difficult only by the RNA extraction kits are performed effectively with the Fruit-mate.

In the case of using in combination with RNAiso Plus, add this product to a homogenized sample and mix well, and then remove the insoluble substance by centrifugation. Add equal amounts of RNAiso Plus to the collected supernatant, then add chloroform and mix well. After separate to 3 layers by centrifugation, collect top liquid layer containing RNA, and recover total RNA by isopropanol precipitation. Thereby, all processes of total RNA extraction are completed in about 1 hour.
In the case of using in combination with the NucleoSpin RNA Plant, add this product to a homogenized sample and mix well, and then remove the insoluble substance by centrifugation. After prepare lysate by adding Lysis Buffer to the collected supernatant, recover total RNA by using the filter cartridge with polymer membrane. It takes about 30 minutes to complete total RNA extraction process.

The plants (its part) below are confirmed with improvement in RNA recovery by using the Fruit-mate:

< Plants (parts) in which the effects of Fruit-mate are observed >
Cherry tomato (fruit), Banana (flesh), Strawberry (fruit), Tomato (seed), Rice (seed), Potato (rhizome), Mandarin orange (peel), Pine (leaf) Aloe (leaf), Mango (fruit), Arabidopsis (seed), Chrysanthemum (leaf), Rose (Leaf).

Note: This product is designed for plants that contain high content of polysaccharide or polyphenol. Depending on types of plant (part), however, use of this product may result in no effect or reduction in recovery of total RNA. Be careful when testing plant (parts) except for those listed above.

II. Components

Fruit-mate for RNA purification 100 ml

III. Storage

Room temperature

- ＊ 2 years from date of receipt under proper storage conditions when unused. Once opened, it should be used earlier avoiding contamination.
- ＊ If this product is kept under 20℃, precipitate might be appeared. In such case, use it after dissolving the precipitate at 37℃.

IV. Materials Required but not Provided

For combination with RNAiso Plus
RNAiso Plus (Cat. #9108/9109)

- Chloroform
- isopropanol
- 75% ethanol
- RNase-free water or TE

For combination with NucleoSpin RNA Plant
NucleoSpin RNA Plant (Cat. #740949.10/.50/.250)

- 1 M or 2 M DTT
- ethanol (70%)
- Special grade ethanol (>99%)
- RNase-free tube (1.5 ml)

V. Guidelines for RNA preparation

1. Sterilized disposable RNase-free plasticware should be used for these experiments. Any plasticware that is not certified RNase-free should be autoclaved before use. When using glass equipment or spatulas, perform dry heat sterilization at 160℃ for at least 2 hours. If dry heat sterilization cannot be performed, treat with 0.1% Diethylpyrocarbonate (DEPC) at 37℃ for 12 hours, then treat with autoclave (prevent RNA's carboxymethylization cause by DEPC) before using it.
2. It is important to separate equipments for RNA experiment exclusively from other equipments.
3. Reagents should be prepared with 0.1% DEPC treated water as much as possible, and treat with autoclave before use. If reagents which are not autoclave treatable are included, use equipments and water which has been sterilized to prepare the solution, and then perform filter sterilization before use.
4. Extra precautions should be taken during the sample preparation, including use of clean disposable gloves and avoiding unnecessary speaking during assembly to prevent RNase contamination from operator sweat or saliva.

VI. Protocols

1. Sample

The plant tissues that are confirmed to have improvement with Fruit-mate are rhizome or fruit tissue (fruit, peel, and seed) which contains large amounts of polysaccharide or polyphenol. The improvement was confirmed in the following plants (parts). Plants not listed below might exhibit no effects or reduce in recovery of RNA.

< Plants (parts) in which the effects of Fruit-mate are observed >
Cherry tomato (fruit), Banana (flesh), Strawberry (fruit), Tomato (seed), Rice (seed), Potato (rhizome), Mandarin orange (peel), Pine (leaf), Aloe (leaf), Mango (fruit), Arabidopsis (seed), Chrysanthemum (leaf), Rose (Leaf).

Use of Fruit-mate for sprout or young leaf, which contains small amounts of polysaccharide or polyphenol, reduce efficiency, so it is recommended to use only RNAiso Plus or NucleoSpin RNA Plant.

2. RNA extraction reagents

Fruit-mate should be used together with RNAiso Plus (Cat. #9108/9109) or NucleoSpin RNA Plant (Cat. #740949.10/.50/.250). Compatibility is not confirmed with other RNA extraction kits.

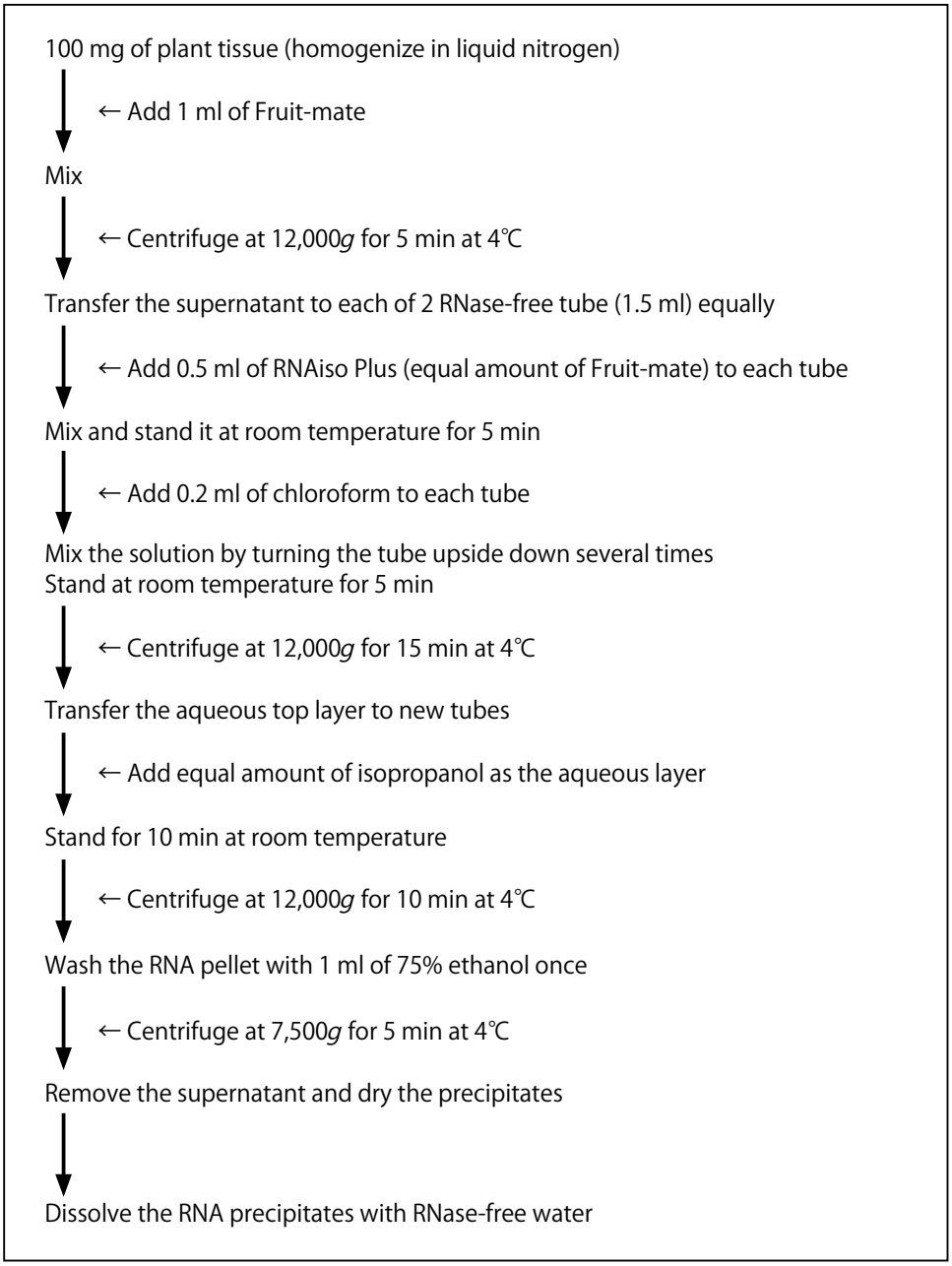
3. Example

Protocol 1: with RNAiso Plus

1. Transfer more than 100 mg of plant tissue to a mortar and homogenize in liquid nitrogen.
2. Add 100 mg of homogenized plant tissue to 1.5 ml RNase-free tube which is cooled in liquid nitrogen. Add 1 ml of Fruit-mate, mix well the homogenized tissue and Fruit-mate. Immediately centrifuge at 12,000*g* for 5 minutes at 4℃ . Transfer and divide equally the supernatant into two new 1.5 ml RNase-free tubes.
3. In each tube, add 0.5 ml of RNAiso Plus (equal amount of the Fruit-mate). Mix and keep at room temperature for 5 minutes.
4. Add 0.2 ml of chloroform to each tube, and mix vigorously by turning the tube upside down, then stand at the room temperature for 5 minutes. Centrifuge at 12,000*g* for 15 minutes at 4℃. By centrifuging, it will separate into three layers; aqueous top layer (contains RNA), middle layer, and bottom organic solvent layer.
5. Transfer the top layer into new centrifuge tube.
   Note: Never touch to middle layer.
6. Add same amount of isopropanol as the aqueous top layer in the centrifuge tube. Mix and stand at room temperature for 10 minutes. Centrifuge at 12,000*g* for 10 minutes at 4℃. RNA will be precipitated at the bottom of the tube.
7. Remove the supernatant and wash the RNA precipitate with 1ml of 75% ethanol once. Centrifuge at 7,500*g* for 5 minutes at 4℃.
8. Remove the supernatant and leave the precipitate.
9. After drying the precipitate at room temperature, dissolve it with adequate amount of the RNase-free water.

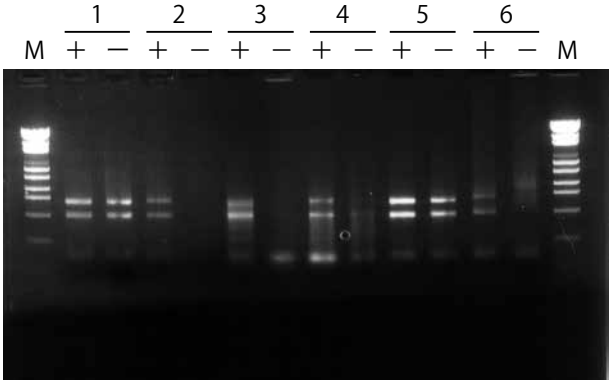
   Note: Do not dry by centrifugation or heating as it might become difficult to dissolve RNA.

Flow chart 1 (Protocol 1 : with RNAiso Plus)



Result 1 (Protocol 1: with RNAiso Plus)

From plant tissue listed below, total RNA extraction was performed by following the Protocol.



M : λ EcoT14 I digest
1 : Cherry tomato (fruit)
2 : Banana (flesh)
3 : Tomato (seed)
4 : Rice (seed)
5 : Potato (rhizome)
6 : Mandarin orange (peel)
＋ : Fruit-mate treatment
－ : Fruit-mate non-treatment

Figure 1. Electrophoresis of total RNA extracted with RNAiso Plus after Fruit-mate treatment (＋) /non-treatment (－)

Protocol 2: with NucleoSpin RNA plant

1. Transfer more than 100 mg of plant tissue to a mortar and homogenize in liquid nitrogen.
2. Add 50 mg of homogenized plant tissue into a 1.5 ml RNase-free tube that was cooled in liquid nitrogen. Add 500 μl of the Fruit-mate, and mix well the homogenized tissue and the Fruit-mate.
3. Centrifuge immediately at 12,000*g* for 5 minutes at 4℃ . Transfer and divide 100 μl of the supernatant to two new 1.5 ml RNase-free tubes.
4. In each tube, add 350 μl of Buffer RA1 (+DTT) or Buffer RAP(+DTT), and vortex for 10 seconds.
5. Set NucleoSpin Filter (violet ring) in a Collection Tube (2 ml), apply the mixture, and centrifuge for 1 minute at 11,000*g*.
6. Discard the NucleoSpin Filter and add 350 μl ethanol (70 %) to the filtrated solution and mix by pipetting up and down. For each preparation take one NucleoSpin RNA Plant Column (light blue ring) placed in a Collection Tube and load the mixture. Centrifuge for 30 seconds at 11,000*g*.
7. Transfer the column in a new Collection Tube (2 ml) and add 350 μl MDB (Membrane Desalting Buffer) and centrifuge at 11,000*g* for 1 minute at room temperature.
8. Apply 95 μl DNase mixture directly onto the center of the silica membrane in the column. Incubate at room temperature(20 - 25℃) for 15 minutes.
9. Add 200 μl Buffer RAW2 to the NucleoSpin RNA Plant Column. Centrifuge for 30 seconds at 11,000*g* at room temperature.
10. Transfer the column into a new Collection Tube and add 700 μl Buffer RA3* to the NucleoSpin RNA Plant Column. Centrifuge for 30 seconds at 11,000*g* at room temperature.
    * It is recommended to use 700 μl of Buffer RA3 in this step though the volume of Buffer RA3 is 600 μl in the protocol of NucleoSpin RNA Plant.
11. Discard flow-through and place the column back into the Collection Tube. Add 250 μl Buffer RA3 to the NucleoSpin RNA Plant Column. Centrifuge for 2 min at 11,000 *g* at room temperature.
12. Transfer the column into a nuclease free Collection Tube (1.5 ml, supplied). Elute RNA in 60 μl RNase-free H_2O (supplied) and centrifuge at 11,000*g* for 1 minute at room temperature.
13. Complete total RNA recovery. If total RNA is not used immediately, store at -20℃ or -80℃.

Flow chart 2 (Protocol 2 : with NucleoSpin RNA Plant)

50 mg of plant tissue (homogenize with liquid nitrogen)

↓ ← Add 500 μl of Fruit-mate

Mix

↓ ← Centrifuge at 12,000*g* for 5 min at 4℃

Transfer the supernatant to each 100 μl of new RNase-free microtube (1.5 ml) equally

↓ ← Add 350 μl of RA1(+DTT) or RAP(+DTT) to each tube and vortex
← Apply the mixture in NucleoSpin Filter (violet ring) in a Collection Tube (2 ml)
← Centrifuge at 11,000*g* for 1 min at room temperature

Discard the NucleoSpin filter (Transfer the supernatant to new 1.5 ml microtube in the presence of visible pellet .)

↓ ← Add 350 μl of ethanol (70%) to the filtrated solution and mix by pipetting up and down
← For each preparation take one NucleoSpin RNA Plant Column (light bue ring) placed in a Collection Tube
← Centrifuge at 11,000*g* for 30 sec at room temperature

Transfer NucleoSpin RNA Plant Column in a new Collection Tube

↓ ← Add 350 μl MDB (Membrane Desalting Buffer)
← Centrifuge at 11,000*g* for 1 min at room temperature

Discard flowthrough

↓ ← Apply 95 μl DNase mixture directly onto the center of NucleoSpin RNA Plant Column (light bue ring).

Incubate for 15 min at room temperature

↓ ← Add 200 μl of RAW2
← Centrifuge at 11,000*g* for 30 sec at room temperature

Transfer NucleoSpin RNA Plant Column (light bue ring) in a new Collection Tube

↓ ← Add 700 μl of RA3
← Centrifuge at 11,000*g* for 30 sec at room temperature

Discard flowthrough

↓ ← Add 250 μl of RA3
← Centrifuge at 11,000*g* for 2 min at room temperature

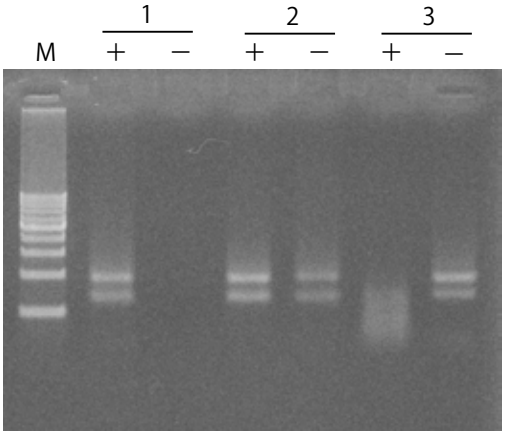
Transfer NucleoSpin RNA Plant Column (light bue ring) in a new 1.5 ml tube

↓ ← Add 60 μl of RNase-free H_2O
← Centrifuge at 11,000*g* for 1 min at room temperature

Collect total RNA

Result 2 (Protocol 2: with NucleoSpin RNA Plant)

From plant tissues listed below, total RNA was extracted by following the Protocol 2.



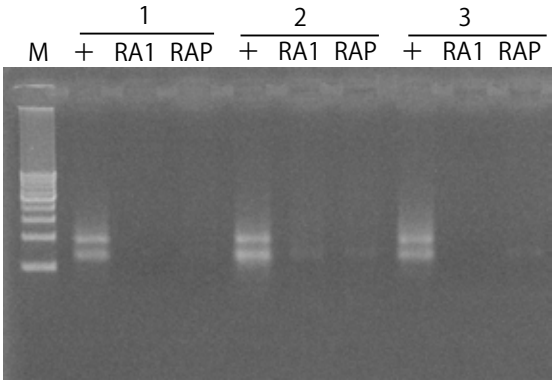
M : 1 kb Ladder
1 : Banana (flesh)
2 : Cherry tomato (fruit)
3 : Hypsizygus marmoreus*
＋ : Fruit-mate treatment (Use of RA1)
− : Fruit-mate non-treatment (Use of RA1)

* The effect of Fruit-mate was not shown in *H. marmoreus*

Figure 2. Electrophoresis of total RNA extracted with NucleoSpin RNA Plant after Fruit-mate treatment (＋) /non-treatment (−)

Result 3 (Protocol 2: with NucleoSpin RNA Plant)

From plant tissues listed below, total RNA was extracted by following the Protocol 2.



M : 1 kb Ladder
1 : Potato (rhizome)
2 : Chrysanthemum (Leaf)
3 : Rose (Leaf)*
＋ : Fruit-mate treatment (Use of RA1)
RA1 : Fruit-mate non-treatment (Use of RA1)
RAP : Fruit-mate non-treatment (Use of RA1)

Figure 3. Electrophoresis of total RNA extracted with RA1 or RAP after Fruit-mate treatment (＋) /non-treatment (RA1, RAP)

VII. Troubleshooting

1. Low yield of RNA

- Homogenization of sample is not adequate
  Homogenize sample until it became small powder particles.
- RNA quantity in tissue is low
  It is known to contain less RNA in some plant tissue. There are reports that root tissue, for instance, contains less RNA than other plant tissues. In addition, mature leaf contains less RNA than young leaf generally. Examples of several plant tissues in below table seem to contain more polysaccharide and polyphenol.

Tissue	Sample amount	RNA Yield (RNAiso Plus)	RNA Yield (NucleoSpin RNA Plant)
Cherry tomato fruit	50 mg	10 - 15 μg	3 - 4 μg
Banana flesh	50 mg	10 - 15 μg	3 - 4 μg
Tomato seed	50 mg	10 - 20 μg	—
Rice	50 mg	5 - 10 μg	—
Potato	50 mg	5 - 10 μg	10 - 15 μg
Orange peel	50 mg	5 μg	—
Chrysanthemum	50 mg	—	50 μg
Rose	50 mg	—	50 μg

— : not tested

- The amount of polysaccharide or polyphenol in sample is low
  Fruit-mate is designed for plant tissues, which contains large quantities of polysaccharide or polyphenol. Refer 1. Sample at VI. Protocols with regard to plant (parts) that showed its effect.
  If Fruit-mate is used for tissues from which RNA can be efficiently extracted by using only RNAiso Plus or only NucleoSpin RNA Plant, recovery of RNA might result in lower.
- Other
  See troubleshooting in the protocols of RNAiso Plus and NucleoSpin RNA Plant.

2. Low purity of RNA

- When amounts of Fruit-mate and RNAiso Plus or Lysis Buffer of in NucleoSpin RNA Plant are smaller than amounts corresponding to tissue sample, protein denaturation might become insufficient.
- Dissolve completely RNA at final step of the extraction (for RNAiso Plus). When the RNA is dried too much after washing with 75% ethanol, dissolving of RNA become difficult. Do not dry with heating or for long time excessively. If leaving on ice for several hours after heating at 60℃ for 5 min, the RNA might be dissolved.
- See troubleshooting in the protocols of RNAiso Plus and NucleoSpin RNA Plant.

3. RNA degradation

- RNA should be extracted from either freshly collected sample or sample frozen immediately with liquid nitrogen and stored at -80℃.
- The reagents and equipments used for RNA extraction may be contaminated with RNase.
- See troubleshooting in the protocols of RNAiso Plus and NucleoSpin RNA Plant.

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4. DNA contamination
   - In using with RNAiso Plus, the amount of RNAiso Plus may be a little. Consider to add quantity recommended.
   - The tissue sample might contain large quantities of organic solvent (ethanol, isopropanol, etc), high concentration buffer, or alkaline solvent.
   - When extracted RNA contains DNA, it is recommended to treat RNA with Recombinant DNase I (RNase-free) (Cat. #2270A/B).
   - See troubleshooting in the protocols of RNAiso Plus and NucleoSpin RNA Plant.

VIII. References

1) J Chirgwin, *et al*. "Isolation of Biologically Active Ribonucleic Acid from Sources Enriched in Ribonuclease". *Biochemistry*. (1979) **18** (24): 5294-5299.
2) D Wallace. "Large-and Small-Scale Phenol Extractions". *Methods in Enzymology*. (1987) **152**: 33-41.
3) Coombs L M, Pigott D, Proctor A, Eydmann M, Denner J, and Knowles M A. "Simultaneous Isolation of DNA, RNA, and Antigenic Protein Exhibiting Kinase Activity from Small Tumor Samples Using Guanidine Isothiocyanate". *Anal Biochem*. (1990) **188**: 338-343.
4) Nicolaides N C and Stoeckert Jr C J. "A Simple, Efficient Method for the Separate Isolation of RNA and DNA from the Same Cells". *Biotechniques*. (1990) **8**: 154-156.
5) J R Feramisco, *et al*. *Molecular Cloning*: 194-195. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
6) Raha S, Merante F, Proteau G, and Reed J K. "Simultaneous Isolation of Total Cellular RNA and DNA from Tissue Culture Cells Using Phenol and Lithium Chloride". *Gene Anal Techn*. (1990) **7**: 173-177.

IX. Related Products

NucleoSpin RNA Plant (Cat. #740949.10/.50/.250)
RNAiso Plus (Cat. #9108/9109)
Recombinant DNase I (RNase-free) (Cat. #2270A/B)

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