Cat. # 9769

For Research Use

TaKaRa

TaKaRa MiniBEST Plant RNA Extraction Kit

Product Manual

v201309Da

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I. Description

TaKaRa MiniBEST Plant RNA Extraction Kit is designed for the rapid, small-scale preparation of highly pure total RNA from plant cells and tissues. The procedure provides a unique cell lysis buffer to achieve isolation of RNA rapidly and conveniently. Total RNA can be isolated from a variety of ordinary plant tissues (e.g., leaves, stems, seedlings) and plant tissues rich in polysaccharide and polyphenols (e.g., fruits, seeds) and fungi with the kit. The kit can effectively isolate RNA longer than 200 bases. In addition, total RNA including small RNA is purified by optional protocol.

The protocol provides a simple method to achieve the rapid isolation of highly purified RNA and the entire procedure can be accomplished within 30 minutes after the tissues or cells being lysed and homogenized. The procedure avoids the phenol and chloroform extraction. The purified RNA contains no protein and no genomic DNA contamination. Up to dozens of microgram of highly purified RNA from 50 - 100 mg plant tissues can be efficiently purified by this kit. The high-quality RNA can be used in Northern blotting, dot blotting, mRNA purification, in vitro translation, RNase protection assays, RT-PCR、Real Time RT-PCR, cDNA library construction and other molecular experiments.

II. Components (50 reactions)

The kit contains Part I and Part II.

Part I (stored at -20℃)

50X DTT Solution	700 μl
Recombinant DNase I (RNase-free)(5 U/μl)	1,000 U
10X DNase I Buffer	1 ml

Part II (stored at room temperature (15 - 25℃))

Buffer PE	28 ml
Buffer NB	1.5 ml x 2
Buffer RL*1,2	28 ml
Buffer RWA*2	28 ml
Buffer RWB*3	30 ml
RNase Free dH_2O	15 ml
RNA Spin Column	50
RNase Free Collection Tube (1.5 ml)	50

*1 : Before operation, add appropriate volume of 50X DTT (Dithiothreitol) Solution into Buffer RL. Add 20 μl 50X DTT Solution per 1 ml of Buffer RL. It is better to prepare the solution before use. This mixture can be stored at room temperature for one month.

*2 : Contain strong denaturant. Be careful to avoid contacting with the skin and eyes. In the case of such contact, wash immediately with plenty water and seek medical advice.

*3 : Before the first use of the kit, add 70 ml of 100% ethanol to Buffer RWB.

Reagents should be supplied by users

1. 100% ethanol
2. 80% ethanol (prepared by 0.1% DEPC treated distilled water)

III. Storage and Shipping

1. Part I should be shipped in the presence of Blue ice and stored at -20℃ .
2. Part II can be stored and shipped at room temperature (15 - 25℃).

IV. Precautions of preventing RNase contamination

Important aspects in the isolation of RNA are to inhibit RNase present in cells and prevent the contamination of RNase present in all instruments and reagents. Consequently, the following precautions should be adopted : wear clean disposable latex gloves ; operate the protocol of isolation of RNA on the exclusive experiment area; avoid talking in the operation and so on. By these precautions, the RNase present in the sweat and saliva of the operator can be minimized.

- Please use disposable plastic ware in the protocol. If used glassware, treat them according to the following protocol :

  Treat the glassware at 37℃ for 12 hours by incubation in water containing 0.1% DEPC(diethyl pyrocarbonate). Then in order to remove the residual DEPC, autoclave at 120℃ for 30 minutes.

- Prepare the reagent :

  The glassware to prepare the reagent used in the RNA isolation should be treated by hot-air sterilization method or the above method. Disposable plastic ware alternatively can be used in the RNA isolation. The distilled water used in the reagent should be treated with 0.1% DEPC and then sterilize by autoclave.
  The special reagent and distilled water used for RNA isolation should not be used mix to avoid cross contamination.

V. Precautions before use

1. If a precipitate appear in Buffer RL, warm it at 60℃ to dissolve the precipitate. Use it after cooling down to room temperature.
2. Add appropriate volume of 50X DTT (Dithiothreitol) Solution into Buffer RL to a final concentration of 2% before use. Namely add 20 μl of 50X DTT Solution into 1 ml of Buffer RL. It should be prepared immediately before use. The mixture can be stored at room temperature for 1 month.
3. If a precipitate appear in lysis Buffer PE, please warm it at 37℃ to dissolve the precipitate. Use it after cooling down to room temperature.
4. Before the first use of the kit, add 70 ml of 100% ethanol to Buffer RWB, and mix well.
5. The maximal loading capacity of RNA Spin Column is 600 μl. If larger volumes are to be processed, load the sample to the column for several times.
6. All steps of the protocol should be carried out at room temperature if there is no special instruction.

VI. Protocol

High quality RNA can be isolated from various plant tissues according to the Protocol-I and Protocol-II. Select the optimal protocol for different tissue based on the characteristic of the sample. Table 1 shows the optimal isolation protocol for different sample.
If the sample is not in the Table 1, perform RNA isolation according to Protocol-I first. If the RNA recovery is low, try to isolate RNA according to Protocol-II.

Materials	Examples	Protocol	
		Protocol-I	Protocol-II
Leaves, Seedlings, Stems of ordinary plant	Tobacco leaves, Arabidopsis, Seedling of herb, Willow leaves	√	
Seedlings and leaves of fruits	Apple seedlings, Banana leaves		√
Leaves of plant rich in polysaccharide and polyphenols	Cypress leaves		√
Materials rich in starch	Plant seeds (maize, rice), Plant tuber (tomato, purple potato)		√
Materials rich in oil	Soybean, Peanut	√	√
Materials rich in sugar	Banana, Mango		√
Materials rich in water	Apple, Fragrant pear	√	
Fungi	Shiitake mushroom, Oyster mushroom	√	

Table 1. The recommended RNA isolation protocol for various plant tissues

1. Lysis of sample

The kit contains two different lysis buffers RL and PE. RNA can be purified from both ordinary plant tissues and plant tissues rich in polysaccharide and polyphenol and difficult for RNA purification. The isolation of total RNA from ordinary plants refers to Protocol-I with 50 - 100 mg of starting amount (the optimal amount is 50 mg). The isolation of total RNA from plants rich in polysaccharide and polyphenols refers to Protocol-II with 20 - 50 mg of starting amount (optimal amount is 50 mg).

Protocol-I : Lysis of ordinary plant tissues

1. Weigh the fresh or frozen plant tissues and place it into a mortar pre-cooled by liquid nitrogen. Then immediately freeze in a small volume of liquid nitrogen. Grind the tissue to a fine powder in continuous presence of liquid nitrogen. Transfer the powder (50 - 100 mg) into a liquid-nitrogen cooled RNase-free 1.5 ml tube. Add 450 μl Buffer RL (make sure that 50X DTT Solution has been added to the Buffer RL). Pipet up and down until the lysate has no precipitate.
2. Centrifuge at 12,000 rpm for 5 minutes at 4℃ .
3. Transfer the supernatant to a new sterile 1.5 ml tube carefully.
4. Proceed to VI-2. Purification of total RNA.

Protocol-II : Lysis of plant tissues rich in polysaccharide and polyphenols

1. Weigh the fresh or frozen plant tissue and place it into a mortar pre-cooled by liquid nitrogen. Then immediately freeze in a small volume of liquid nitrogen. Grind tissue to a fine powder in the continuous presence of liquid nitrogen. Transfer the powder (20 - 50 mg) into a liquid-nitrogen cooled RNase-free 1.5 ml tube. Add 450 μl Buffer PE. Pipet up and down until the lysate has no precipitate.
2. Centrifuge at 12,000 rpm for 5 minutes at 4℃ .
3. Transfer the supernatant to a new sterile 1.5 ml tube carefully. Add 1/10 volume Buffer NB (a precipitate may appear), vortex to mix well.
4. Centrifuge at 12,000 rpm for 5 minutes at 4℃ .
5. Transfer the supernatant to a new 1.5 ml tube carefully. Add 450 μl Buffer RL (make sure that 50X DTT Solution has been added to the Buffer RL). Pipet up and down to mix well.
6. Proceed to VI-2. Purification of total RNA.

2. Purification of total RNA

1. Add 1/2 volume (Lysate or mixture) of 100% ethanol (a precipitate may appear). Pipet up and down to mix well.
2. Immediately apply the mixture (including any precipitate) to RNA Spin Column in a 2 ml collection tube. (If the volume of the mixture more than 600 μl, the mixture should be divided into several times. But do not load the mixture more than 600 μl at each time.)
3. Centrifuge at 12,000 rpm for 1 minute. Discard the flow-through. Place the RNA Spin Column back into the 2 ml collection tube.
4. Add 500 μl Buffer RWA to RNA Spin Column. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
5. Add 600 μl Buffer RWB to RNA Spin Colum. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through. Take care to add Buffer RWB along the tube wall of the RNA Spin Column to completely remove the residual salt.

   NOTE : Make sure that the indicated 100% ethanol is added to Buffer RWB.
6. DNase I Digestion (optional)
   By using RNA Spin Column, the most genomic DNA in the plant tissues can be effectively removed. If the downstream experiment strictly requires the high quality of RNA, genomic DNA digestion may be carried out by adding DNase I to the membrane of the Column.
   (1) Prepare the DNase I mixture : in a 1.5 ml microtube, add 5 μl 10X DNase I Buffer, 4 μl Recombinant DNase I (RNase -free)(5 U/μl), and 41 μl RNase free dH_2O. Mix gently by inverting the tube.
   (2) Apply 50 μl DNase I mixture directly onto the center of the membrane of the column. Incubate at room temperature for 15 minutes.
   (3) Add 350 μl Buffer RWB to the RNA Spin Column. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
7. Repeat step 5.
8. Place the RNA Spin Column back into the 2 ml Collection Tube. Centrifuge at 12,000 rpm for 2 minutes.

9. Place the RNA Spin Column in a new 1.5 ml RNase Free Collection Tube (supplied). Add 50 - 200 μl RNase Free dH_2O or 0.1% DEPC treated water directly to the Spin Column. Incubate at room temperature for 5 minutes.
10. Centrifuge at 12,000 rpm for 2 minutes to elute the RNA.
11. If the yield of the RNA is lower, elute again with additional 50 - 200 μl RNase Free dH_2O or 0.1% DEPC water to the Spin Column to increase the yield. If higher RNA concentration is required, re-apply the elution from step 10 back to the Spin Column. Incubate at room temperature for 5 minutes. Centrifuge at 12,000 rpm for 2 minutes to elute the RNA.

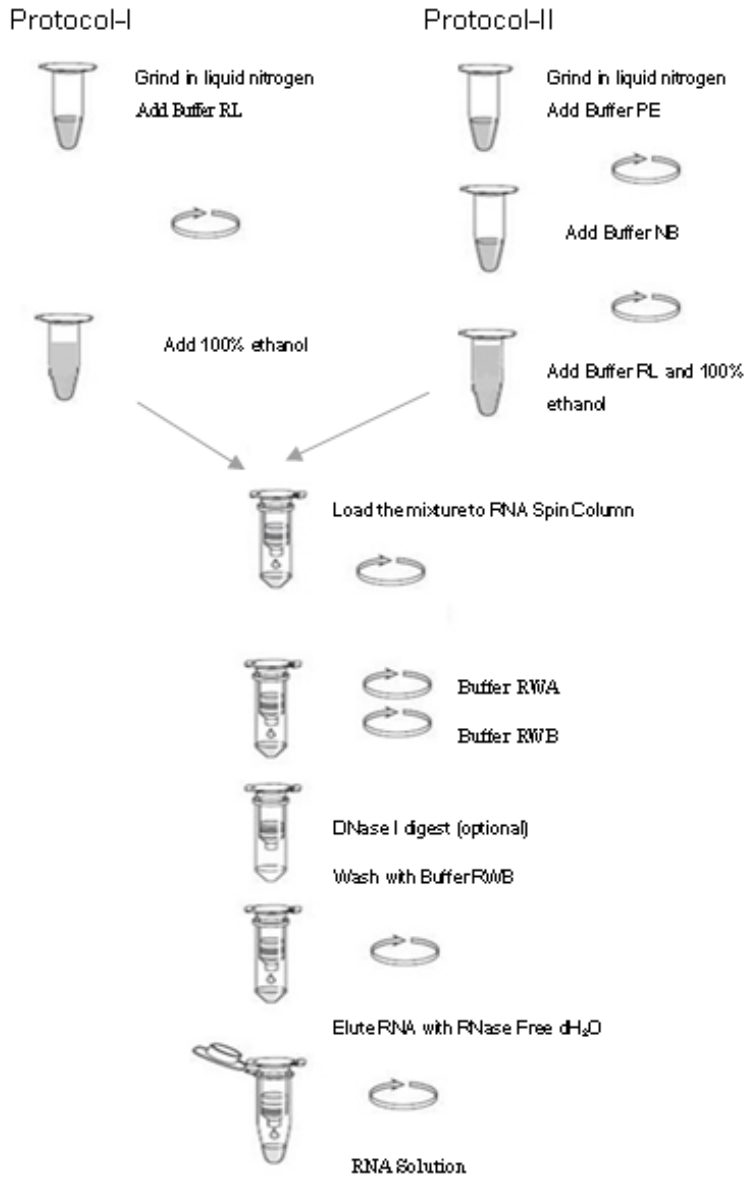


Figure 1. Flow chart

VII. Yields of Total RNA from various materials

Sample	RNA Yield
Soybean seeds	40 - 50 μg /50 mg
Peanut seeds	20 - 25 μg/50 mg
Maize seeds	10 - 15 μg/100 mg
Banana fruits	2 - 3 μg/50 mg
Apple fruits	1 - 2 μg/50 mg
Potato tubers	10 - 15 μg/50 mg
Purple potato tubers	4 - 5 μg/50 mg
Oyster mushroom (Fungi)	10 - 15 μg/50 mg
Shiitake mushroom (Fungi)	10 - 15 μg/50 mg
Ginkgo leaves	10 - 15 μg/50 mg
Maize leaves	30 - 40 μg/100 mg
Arabidopsis thaliana leaves	10 - 15 μg/100 mg
Tobacco leaves	20 - 30 μg/100 mg
Willow leaves	40 - 50 μg/100 mg
Cypress leaves	20 - 30 μg/100 mg

Table 2. Yields of total RNA from various materials

Table 2 shows the yields of total RNA from various materials. RNA content depends on the growing state and freshness of the plants, so this table is showing as reference.

VIII. Appendix (Purification of total RNA containing small RNA)

RNA molecule longer than 200 bases can be efficiently obtained according to the above protocol. However, the recovery of small RNA shorter than 200 bases is relatively lower. If more content of small RNA is required, the following protocol is recommended to obtain RNA containing more small RNA. But the yield of total RNA will be lower by using this method.

1. Lysis of sample

The method of lysis of the sample is same as the "VI-1. Lysis of sample". Then proceed to Purification of total RNA according to the following protocol.

NOTE : Prepare 80% ethanol (prepared by 0.1% DEPC treated water) before start.

2. Purification of total RNA rich in small RNA

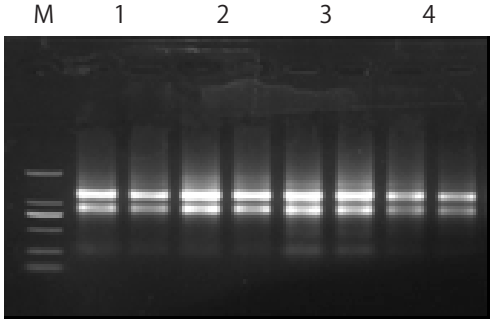
1. Add 1.5 volume of 100% ethanol (a precipitate may appear). Pipet up and down to mix well.
2. Load the mixture (including any precipitate) to RNA Spin Column in a 2 ml collection tube. (If the volume of the mixture is more than 600 μl, the mixture should be divided into several times. But do not load the mixture more than 600 μl at each time.)
3. Centrifuge at 12,000 rpm for 1 minute. Discard the flow-through. Place the RNA Spin Column back into the 2 ml collection tube.

4. Add 600 μl 80% ethanol to RNA Spin Column. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
   Note: Add 80% ethanol along the tube wall of the RNA Spin Column to completely remove the residual salt.
5. DNase I Digestion (optional)
   By using RNA Spin Column, the most genomic DNA in the plant cells can be effectively removed. If the downstream experiment strictly requires high quality of RNA, genomic DNA digestion may be carried out by adding DNase I to the membrane center of the Column.
   (1) Prepare the DNase I mixture : in a 1.5 ml microtube, add 5 μl 10X DNase I Buffer, 4 μl Recombinant DNase I (RNase -free), and 41 μl RNase free dH_2O. Mix gently by inverting the tube.
   (2) Apply 50 μl DNase I mixture directly onto the center of the membrane of the column. Incubate at room temperature for 15 minutes.
   (3) Add 350 μl Buffer RWB to the RNA Spin Column. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
6. Repeat the step 4.
7. Place the RNA Spin Column back into the 2 ml Collection Tube. Centrifuge at 12,000 rpm for 2 minutes.
8. Place the RNA Spin Column in a new 1.5 ml RNase Free Collection Tube (supplied). Add 30 - 100 μl RNase Free dH_2O or 0.1% DEPC water directly to the Spin Column. Incubate at room temperature for 5 minutes.
9. Centrifuge at 12,000 rpm for 2 minutes to elute the RNA.
10. If the yield of the RNA is lower, elute again with additional 30 - 100 μl RNase Free dH_2O or 0.1% DEPC water to the Spin Column to increase the yield. If higher RNA concentration is required, re-apply the elution from step 9 back to the Spin Column. Incubate at room temperature for 5 minutes. Centrifuge at 12,000 rpm for 2 minutes to elute the RNA.

IX. Experimental Examples

1. Purification of total RNA from seedlings

Dozens of microgram of highly purified total RNA can be obtained from 100 mg seedlings according to the Protocol-I. Electrophoresis is shown in Figure 2.

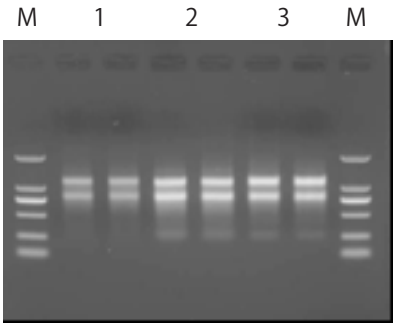


M : DL2,000 DNA Marker
1 : Broomcorn seedling
2 : Maize seedling
3 : Mung bean seedling
4 : Sunflower seedling

Figure 2. Total RNA of seedlings

2. Purification of total RNA from plant seeds

Dozens of microgramme of highly purified total RNA can be obtained from 50 mg plant seeds according to the Protocol-II. Electrophoresis is shown in Figure 3.

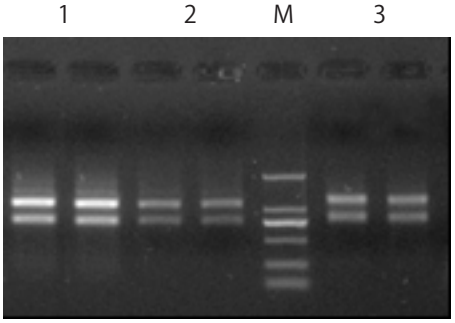


M : DL2,000 DNA Marker
1 : Peanut seed
2 : Sunflower seed
3 : Maize seed

Figure 3. Total RNA of plant seeds

3. Purification of total RNA from plant fruits

Dozens of microgram of highly purified total RNA can be obtained from 100 mg plant fruits according to the Protocol-I. Electrophoresis is shown in Figure 4.

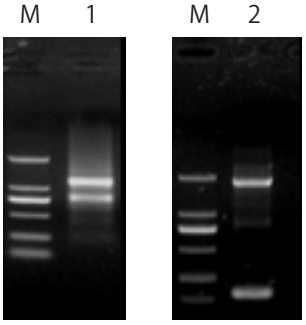


M : DL2,000 DNA Marker
1 : Fragrant pear fruit
2 : Apple fruit
3 : Tomato fruit

Figure 4. Total RNA of plant fruits

4. Application of purified total RNA

Total RNA isolated from tobacco leaves was proceeded 5'-RACE analysis. 1.8 kb target DNA fragment was obtained. Electrophoresis is shown in Figure 5.



M : DL2,000 DNA Marker
1 : Tobacco leaves RNA
2 : 1.8 kb DNA fragment obtained by 5'RACE kit

Figure 5. 5'-RACE analysis

X. Troubleshooting

Q1 : When yield of total RNA is low

A1 : Using TaKaRa MiniBEST Plant RNA Extraction Kit, please refer to Table 2 for the yields of total RNA from different tissues or cells to purify total RNA. If the yield of total RNA is low, perform the following:

(1) Insufficient disruption and homogenization. It is essential to lyse the sample completely. See details on VI-1. Lysis of sample in protocols.
(2) Too much starting material. The optimal amount of starting material for ordinary plant tissue is 50 - 100 mg, and that for the plant tissue rich in polysaccharide and polyphenols is 20 - 50 mg.
(3) RNA elution insufficient. We recommend to elute RNA once again. Incubation time after adding RNase-Free dH_2O or 0.1% DEPC water to the Spin Column can prolong up to 10 minutes.
(4) Elution contains residual ethanol. During the second wash with Buffer RWB, be sure to centrifuge at 12,000 rpm for 1 minute to dry RNA Spin Column membrane. Then we recommend to perform another 2 minutes centrifugation as described in the protocols. Residual ethanol will decrease the yield of RNA.

Q2 : When the purified RNA is degraded.

A2 : (1) Sample are not fresh. Use fresh samples as much as possible, or freeze the samples in liquid nitrogen and then store at -80℃ .
(2) There is RNase in the reagents or materials. Before starting the protocol, see "IV. Precautions of preventing RNase contamination" thoroughly.
(3) The sample is rich in RNase. It is recommended to use less starting material and increase the volume of Lysis Buffer for tissues or cells rich in RNase.

Q3 : When the purified RNA contain genomic DNA contamination.

A3 : By on-column digestion with DNase I, DNA contaminations can be effectively removed. If RNA is contaminated with genomic DNA, perform the following.
(1) Insufficient disruption and homogenization. It is essential to lyse the sample completely. See details on VI-1. Lysis of sample in the protocols.
(2) Samples rich in genomic DNA. Genomic DNA content is different in different tissues. For some materials rich in genomic DNA, It is recommended to use less starting material and increase the volume of Buffer RL/Buffer PE or the volume of DNase I.
(3) DNase digestion with DNase I is omitted. If the downstream experiment has a strictly require on high quality of RNA, or the samples are rich in genomic DNA, DNase digestion should be carried out according to the protocols.

Q4 : What is the meaning of the absorbance values of RNA?

A4 : The absorbance values at 260 nm (A_{260}), 320 nm (A_{320}), 230 nm (A_{230}), and 280 nm (A_{280}) represent the concentration of nucleic acid, background (turbidity of reagent), concentration of salt and protein, respectively. The ratio of OD_{260}/OD_{280} represents the degree of contamination of organics such as protein and so on. The value should be between 1.8 and 2.2 for high quality RNA, the ratio lower than 1.8 means that the contamination of protein is obvious, and the value higher than 2.2 means that the RNA was degraded to mononucleotide.
Dilute the RNA sample using TE buffer when checking the RNA absorbance values.

Q5 : How to measure RNA concentration.

A5 : Count the concentration of RNA according the absorbance of RNA:
RNA (μg/μl) = (OD_{260}-OD_{320}) x dilution factor x 0.04 μg/μl

Q6 : How integrity of RNA extracted by the kit?
A6 : Using Takara MiniBEST Plant RNA Extraction Kit, RNA molecule longer than 200 bases can be efficiently purified. If small RNA is required, please refer to the Appendix about protocol of purification of RNA containing small RNA, whereas the yield of total RNA will be lower.

Q7 : How to estimate the purification protocol of some material?
A7 : It is recommended to select the optimal protocol refer to Table 1. If the sample is not in Table 1, we recommend to try to extract RNA according to Protocol-I first. If RNA recovery is low, try to isolate of RNA according to Protocol-II.