Cat. # 9767

For Research Use

TaKaRa

TaKaRa MiniBEST Universal RNA Extraction Kit

Product Manual

v201911Da

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I. Description

TaKaRa MiniBEST Universal RNA Extraction Kit is designed for the rapid, small-scale preparation of highly pure total RNA from cultured cells, animal tissues and plant tissues. The kit use an unique cell lysis buffer to achieve isolation of RNA rapidly and conveniently. The procedure avoids the phenol and chloroform extraction. Cells or tissues are lysed by incubation in the lysis buffer after homogenization or grinding in the presence of liquid nitrogen. With the gDNA Eraser Spin Column (for remove the genome DNA) and RNA Spin Column (for binding RNA), highly pure and quality RNA can be obtained.
The protocol provides a simple method to achieve the rapid isolation of highly pure RNA and the entire procedure can be accomplished within 20 minutes after tissues or cells being lysed. The pure RNA obtained by the kit basically contain no protein and have no genomic DNA contamination. The protocol allows the purification of about 10 - 30 mg of high quality of RNA from 1.0 x 10^5 - 1.0 x 10^7 cultured cells , 5 - 40mg of mammalian tissues, and 50 - 100 mg plant tissues. The highly pure RNA eluted with RNase-free H_2O can be used in Northern blot, dot blot, mRNA purification, in vitro translation, RNase protection assay, RT-PCR, real time RT-PCR, cDNA library construction and other kinds of molecular experiments.
Using the gDNA Eraser Spin Column can isolate easily genome DNA which can be used in PCR reaction and other kinds of molecular experiments*1.

*1 For gDNA extraction, follow the gDNA Extraction Protocol in the Appendix.

II. Components (50 reactions)

The kit contains Part I and Part II.

■ Part I

50X DTT Solution	700 µl
Recombinant DNase I (RNase-free ; 5 U/µl)	1000 U
10X DNase I Buffer	1 ml

■ Part II

Buffer RL*2	32 ml
Buffer RWA*2	28 ml
Buffer RWB*3	30 ml
RNase Free dH_2O	15 ml
gDNA Eraser Spin Column	50
RNA Spin Column	50
Collection Tube (2 ml)	50
RNase Free Collection tube (1.5 ml)	50

*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 not supplied in this kit]

- ◆ 100% ethanol
- ◆ 70% ethanol(prepare by 0.1% DEPC treated distilled water)
- ◆ PBS

III. Storage and Shipping

1. Part I should be shipped and stored at -20℃.
2. Part II can be stored and shipped at room temperature (15 - 25℃).

IV. Precautions for Preventing RNase Contamination

Important aspects in the isolation of RNA is to inhibit RNases present in cells and to prevent contamination of RNases present in all instruments and reagents.
Therefore, 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 precaution, RNases present in the sweat and saliva of the operator can be minimized.
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 for RNA isolation should be treated by hot-air sterilization method or the above method. Disposable plastic ware alternatively can be used for RNA isolation. The distilled water used in the reagent should be treated with 0.1% DEPC and then sterilize by autoclave.
The specific reagent and distilled water used for RNA isolation should not be used mix to avoid cross contamination.

V. Precautions before Starting

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. Before the first use of the kit, add 70 ml of 100% ethanol to Buffer RWB, and mix well.
4. The maximal loading capacity of gDNA Eraser Spin Column and RNA Spin Column are each 600 μl. If larger volume is to be processed, load the sample to the column into several times.
5. All steps of the protocol should be carried out at room temperature if there is no special instruction.

VI. Sample Amount and Buffer RL Volume

The amount of starting material of tissue and cells have a great influence on RNA yield and purity. The yield and quality of RNA will be lower when using more starting material. Generally, RNA can be purified from 1.0 x 10^5 - 1.0 x 10^7 cultured cells, 5 - 40 mg of frozen or fresh mammalian tissues and 50 - 100 mg of frozen or fresh plant tissues. We recommend to use the appropriate amount of cultured cells 1.0 x 10^6, 10 mg for animal tissues, and 50 mg for plant tissues. It is recommended to reduce the amount of starting materials if the tissues, such as spleen, kidney and so on, are rich in genomic DNA. And it is recommended to reduce the amount of starting material and increase the volume of Buffer RL for some plant tissue (stem or root and so on) and some hard mammalian tissue (lung, heart and so on). For optimal recovery, refer to Table 1 for the amount of starting materials of different tissue and the volume of lysis Buffer RL.

Sample Amount	Recommended volume of Buffer RL
Adherent cells (less than 6 cm diameter dish)	350 μl
Adherent cells (6 - 10 cm diameter dish)	600 μl
Less than 5 x 10^6 of suspension cells	350 μl
5 x 10^6 - 10^7 of suspension cells	600 μl
5 - 20 mg of ordinary animal tissue (e.g., brain, liver)	350 μl
20 - 40 mg of ordinary animal tissue (e.g., brain, liver)	600 μl
5 - 20 mg of special tissue (e.g., lung, kidney, spleen)	350 μl
20 - 40 mg of special tissue (e.g., lung, kidney, spleen)	600 μl
50 - 100 mg of plant tissue (leaf, stem)	500 μl

Table 1. Starting amount of different samples and Buffer RL volume

VII. Protocols

● Flow chart

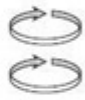
Materials

Grind in Liquid nitrogen or homogenize
Add Buffer RL

Transfer lysate to gDNA Eraser Spin Column

Add equal volume of 70% ethanol to the flow-through

Load the mixture to RNA Spin Column for binding RNA



Buffer RWA
Buffer RWB
} Wash RNA Spin Column Column

DNase I digest (optional)

Buffer RWB wash

Add RNase Free dH_2O to elute RNA

RNA Solution

For cultured mammalian cells

1. Cell lysis:
   - Lysis of suspension cultured mammalian cells
     (1) Centrifuge cell culture of suspension cells at 8,000 *g* at 4℃ for 2 minutes, then discard the supernatant.
     (2) Resuspend the cell pellet with 1X PBS, and centrifuge at 8,000 *g* at 4℃ for 2 minutes, discard the supernatant.
     (3) Add appropriate volume (refer Table 1) of Buffer RL (make sure that 50X DTT Solution has been added to Buffer RL) to the cell pellets.
     (4) Pipet up and down until the lysate has no precipitate.

   - Lysis of adherent cells
     (1) Discard the culture medium then wash the cell with 1X PBS.
     (2) Add appropriate volume (refer Table 1) of Buffer RL (make sure that 50X DTT Solution has been added to Buffer RL) to the adherent cells. Spread the buffer over the surface of the cells then incubate at room temperature for a moment in order to lyse the cells. Scrape off the cells by pipetting (for cells tightly adherent to the culture flask, a cell scraper can be used to scrape off the cells) .
     (3) Transfer the cell lysate to a centrifuge tube, pipet up and down until the lysate has no precipitate.
2. Incubate the cell lysate at room temperature for 2 minutes.
3. Set gDNA Eraser Spin Column in a 2 ml Collection Tube (supplied by the kit).
4. Apply the lysate to gDNA Eraser Spin Column in Collection Tube (2 ml).
5. Centrifuge at 12,000 rpm for 1 minutes.
6. Discard gDNA Eraser Spin Column (do not discard when you isolate genomic DNA. See Appendix). Add equal volume of 70% ethanol to the flow-through in the 2 ml Collection Tube (a precipitate may appear) and mix thoroughly by pipetting up and down.
7. Apply the mixture (including any precipitate) to RNA Spin Column in 2 ml Collection Tube. (If the volume of the mixture is more than 600 μl, the mixture is applied by dividing into several times. Never load the mixture over 600 μl at each time.)
8. Centrifuge at 12,000 rpm for 1 minute. Discard the flow-through. Place the RNA Spin Column back into the 2 ml Collection Tube.
9. Add 500 μl Buffer RWA to RNA Spin Column. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
10. Add 600 μl Buffer RWB to RNA Spin Colum. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
    Note: Make sure that the indicated 100% ethanol has been added to Buffer RWB. Add Buffer RWB along the wall of the RNA Spin Column, it is better to completely remove the residual salt on the wall.
11. DNase I Digest (optional)
    By the gDNA Eraser Spin Column and RNA Spin Column, the most genomic DNA in the cultured cells can be effectively removed. If the downstream experiment has a strictly demand on the purity of RNA, genomic DNA digestion can be carried out by adding DNase I to the silica 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) to 41 μl RNase free dH_2O. Mix gently by inverting the tube.
    (2) Apply 50 μl the DNase I mixture directly onto the center of the silica membrane of the RNA Spin Column. Incubate at room temperature for 15 minutes.
    (3) Add 350 μl RWB to the RNA Spin Column. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.

12. Repeat step 10.
13. Set the RNA Spin Column back in the 2 ml Collection Tube. Centrifuge at 12,000 rpm for 2 minutes.
14. Place the RNA Spin Column in a new 1.5 ml RNase Free Collection Tube (supplied). Add 50 - 200 μl RNase-free water directly to the RNA Spin Column membrane. Incubate at room temperature for 5 minutes.
15. Centrifuge at 12,000 rpm for 2 minutes to elute the RNA.
16. If the yield of the RNA is low, use another 50 - 200 μl RNase-free water to the Spin Column to increase the yield. If high RNA concentration is required, apply the elution from step 15 back to the RNA Spin Column again. Incubate at room temperature for 5 minutes. Centrifuge at 12,000 rpm for 2 minutes to elute the RNA.

For mammalian tissues

1. Tissue lysis
   - Homogenization of mammalian tissues according to one of the following 3 methods.
     (1) Weight the fresh or frozen mammalian tissue and place it into a mortar pre-cooled with liquid nitrogen. Freeze immediately the tissue in a small volume of liquid nitrogen. Grind the tissue to fine powder in the continuous presence of liquid nitrogen. Transfer the powder tissue into a RNase-free 1.5 ml microtube pre-cooled with liquid nitrogen. Add appropriate volume (see Table 1) of 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) Place the weighed (fresh or frozen) tissues into a RNase-free 1.5 ml microtube. Add appropriate volume (see Table 1) of Buffer RL (make sure that 50X DTT Solution has been added to the Buffer RL). Homogenize immediately the tissue by vigorous agitation in the presence of beads and lysis buffer using a tissue homogeneous device. Pipet up and down until the lysate has no precipitate.
     (3) Place the weighed (fresh or frozen) tissue in a suitable sized vessel. Add appropriate volume (see Table 1) of Buffer RL (make sure that 50X DTT Solution has been added to the Buffer RL). Homogenize immediately the tissue using a rotor-stator homogenizer on the ice until it is uniformly homogeneous. Transfer the lysate to a RNase-free 1.5 ml microtube.

   - Homogenization of the mammalian tissues for difficult disruption or rich in genomic DNA.
     Weigh a fresh or frozen mammalian tissue and place it into a mortar pre-cooled with liquid nitrogen. Freeze quickly in a small volume of liquid nitrogen. Grind the tissue to fine powder in the continuous presence of liquid nitrogen. Transfer the powder tissue into RNase-free 1.5 ml microtube pre-cooled liquid nitrogen. Add appropriate volume (see Table 1) of 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.
     Note : Complete disruption and homogenization of the starting material is a key requirement for obtaining of highly quality total RNA. If the lysate is viscosity, it can pass through a 20-gauge needle attached to a sterile plastic syringe at least 5 - 10 times to clear the lysate.

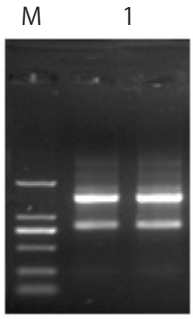
2. Centrifuge at 12,000 rpm for 5 minutes at 4℃.
3. Set a gDNA Eraser Spin Column in a 2 ml Collection Tube (supplied in the kit).
4. Apply the lysate to the gDNA Eraser Spin Column in the 2 ml Collection Tube.
5. Centrifuge at 12,000 rpm for 1 minutes.
6. Discard gDNA Eraser Spin Column (do not discard when you isolate genomic DNA. See Appendix). Add equal volume of 70% ethanol to the flow-through in the Collection Tube (there may appear precipitate) and mix thoroughly by pipetting up and down.
7. Apply 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, repeat the procedure. But do not load the mixture more than 600 μl.)
8. Centrifuge at 12,000 rpm for 1 minute. Discard the flow-through. Set the RNA Spin Column back into the 2 ml Collection Tube.
9. Add 500 μl Buffer RWA to RNA Spin Column. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
10. Add 600 μl Buffer RWB to RNA Spin Colum. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
    Note : Make sure that the indicated 100% ethanol has been added to Buffer RWB. Add Buffer RWB along the wall of the RNA Spin Column, it is better to completely remove the residual salt on the wall.
11. DNase I Digest (optional)
    By the gDNA Eraser Spin Column and RNA Spin Column, most genomic DNA in the tissues can be effectively removed. If the downstream experiment has a strictly demand on purity of RNA, genomic DNA digestion can be carried out by adding DNase I to the silica membrane of the Column.
    (1) Prepare 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) to 41 μl RNase free dH_2O. Mix gently by inverting the tube.
    (2) Pipet 50 μl DNase I mixture directly onto the center of the silica membrane of the column. Incubate at room temperature for 15 minutes.
    (3) Add 350 μl RWB to the RNA Spin Column. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
12. Repeat step 10.
13. Set the Column back into the 2 ml Collection Tube. Centrifuge at 12,000 rpm for 2 minutes.
14. Place the RNA Spin Column in a new 1.5 ml Collection Tube (supplied). Add 50 - 200 μl RNase-free water directly to the Spin Column membrane. Incubate at room temperature for 5 minutes.
15. Centrifuge at 12,000 rpm for 2 minutes to elute the RNA.
16. If yield of the RNA is low, use another 50 - 200 μl RNase-free water to the Spin Column to increase the yield. If high RNA concentration is required, add the elution from Step 15 back to the Spin Column. Incubate at room temperature for 5 minutes. Centrifuge at 12,000 rpm for 2 minutes to elute the RNA.

For plant tissues

1. Weight a fresh or frozen plant tissue and place it into a mortar pre-cooled with liquid nitrogen. Freeze promptly in a small volume of liquid nitrogen. Grind the tissue to fine powder in the continuous presence of liquid nitrogen. Transfer the powder tissue into a RNase-free 1.5 ml microtube pre-cooled with liquid nitrogen. Add appropriate volume (see Table 1) of 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.
   Note : Complete disruption and homogenization of the starting material is a key requirement for obtaining of highly quality total RNA. If the lysate is viscosity, it can pass through a 20-gauge needle attached to a sterile plastic syringe at least 5 - 10 times to clear the lysate.
2. Centrifuge at 12,000 rpm for 5 minutes at 4℃.
3. Set a gDNA Eraser Spin Column in a 2 ml Collection Tube (supplied).
4. Transfer the lysate to the gDNA Eraser Spin Column in 2 ml Collection Tube.
5. Centrifuge at 12,000 rpm for 1 minute.
6. Discard the gDNA Eraser Spin Column (do not discard when you isolate genomic DNA. See Appendix). Add equal volume of 70% ethanol to the flow-through in the 2 ml Collection Tube (there may appear precipitate) and mix thoroughly by pipetting up and down.
7. Apply the mixture (including any precipitate) to a RNA Spin Column in 2 ml Collection Tube. (If the volume of the mixture more than 600 μl, repeat the procedure. But do not load the mixture more than 600 μl.)
8. Centrifuge at 12,000 rpm for 1 minute. Discard the flow-through. Place the RNA Spin Column back into the 2 ml Collection Tube.
9. Add 500 μl Buffer RWA to RNA Spin Column. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
10. Add 600 μl Buffer RWB to RNA Spin Colum. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
    Note : Make sure that the indicated 100% ethanol has been added to Buffer RWB. Add Buffer RWB along the wall of the RNA Spin Column, it is better to completely remove the residual salt on the wall.
11. DNase I Digest (optional)
    By the gDNA Eraser Spin Column and RNA Spin Column, the most genomic DNA in a tissue can be effectively removed. If the downstream experiment has a strictly demand on the purity of RNA, genomic DNA digestion can be carried out by adding DNase I to the silica 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) to 41 μl RNase free dH_2O. Mix gently by inverting the tube.
    (2) Pipet 50 μl DNase I mixture directly onto the center of the silica membrane of the Column. Incubate at room temperature for 15 minutes.
    (3) Add 350 μl RWB to the RNA Spin Column. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
12. Repeat step 10.
13. Set RNA Spin Column back into the 2 ml Collection Tube. Centrifuge at 12,000 rpm for 2 minutes.
14. Place the RNA Spin Column in a new 1.5 ml Collection Tube (supplied). Add 50 - 200 μl RNase-free water directly to the Spin Column membrane. Incubate at room temperature for 5 minutes.
15. Centrifuge at 12,000 rpm for 2 minutes to elute the RNA.
16. If yield of the RNA is low, use another 50 - 200 μl RNase-free water to the Spin Column to increase the yield. If high RNA concentration is required, add the elution from Step 15 back to the Spin Column. Incubate at room temperature for 5 minutes. Centrifuge at 12,000 rpm for 2 minutes to elute the RNA.

VIII. Experimental Examples

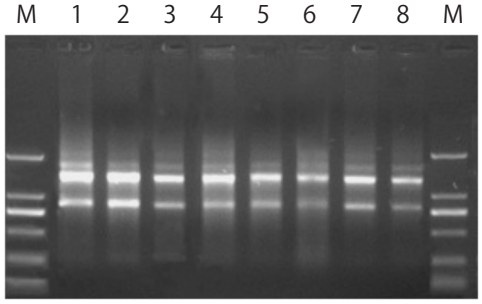
1. Purification of total RNA from cultured cells
   10 μg total RNA can be obtained from 1.0 x 10^6 HL 60 cells using the kit (Figure 1).



M：DL2,000 DNA Marker
1：HL 60 cell RNA

Figure 1. Total RNA of cultured cells

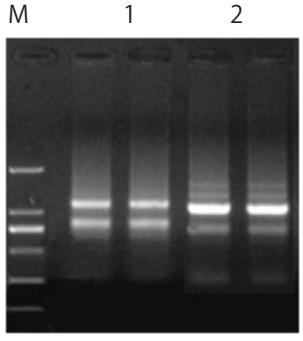
2. Purification of total RNA from mammalian tissues
   Highly pure total RNA can be obtained from 5 - 10 mg of various mouse tissues using the kit (Figure 2).



M : DL2,000 DNA Marker
1 : Mouse Liver RNA
2 : Mouse Heart RNA
3 : Mouse Kidney
4 : Mouse Lung RNA
5 : Mouse Pancreas RNA
6 : Mouse Spleen RNA
7 : Mouse Brain RNA
8 : Mouse Thymus RNA

Figure 2. Total RNA of mammalian tissues

3. Purification of total RNA from plant tissues
   Highly pure RNA can be obtained from 50 mg leaves of maize and willow (Figure 3).



M : DL2,000 DNA Marker
1 : Leaf of maize RNA
2 : Leaf of willow RNA

Figure 3. Total RNA of plant tissues

IX. Yields of Total RNA from Various Materials

Samples		RNA yield
Animal tissues	Mouse Liver	30 - 50 μg/10 mg
	Mouse Heart	5 - 10 μg /10 mg
	Mouse Kidney	20 - 30 μg/10 mg
	Mouse Pancreas	5 - 15 μg/10 mg
	Mouse Spleen	20 - 30 μg/10 mg
	Mouse Thymus	10 - 20 μg/10 mg
	Mouse Lung	10 - 20 μg/10 mg
	Mouse Brain	5 - 10 μg/10 mg
	Rat Muscle	2 - 4 μg/10 mg
Plant Tissues	Leaf of Maize	30 - 40 μg/100 mg
	Leaf of gourd	10 - 15 μg/100 mg
	Leaf of willow	40 - 50 μg/100 mg
	Leaf of mung bean	15 - 20 μg/100 mg
Cultured Cell	HL60 cell	8 - 15 μg/10^6 cells

X. Appendix：Genomic DNA Extraction

Genomic DNA can be simply extracted from gDNA Eraser Spin Column in according to the following protocol.

1. Lyse the sample following Steps 1 to 4 in the VII. Protocols.
2. Set the gDNA Eraser Spin Column back into 2 ml Collection Tube again.
3. Add 500 μl Buffer RWA to the gDNA Eraser Spin Column. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
4. Add 600 μl Buffer RWB to gDNA Eraser Spin Column. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
   Note : Make sure that the indicated 100% ethanol has been added to Buffer RWB. Add Buffer RWB along the wall of the gDNA Eraser Spin Column, it is better to completely remove the residual salt on the wall.
5. Repeat Step 4.
6. Place the gDNA Eraser Spin Column back into the 2 ml Collection Tube. Centrifuge at 12,000 rpm for 2 minutes.
7. Place the gDNA Eraser Spin Column in a new 1.5 ml RNase-free Collection Tube (supplied). Add 50 μl dH_2O or TE Buffer and incubate at room temperature for 5 minutes.
8. Centrifuge at 12,000 rpm for 2 minutes to elute the genomic DNA.

XI. Troubleshooting

Q1: Why is a RNA Spin Column clogged?
A1: The reasons are almost the following.
(1) Incomplete lysis of samples. The incomplete lysis of the sample may result in the block of RNA Spin Column. See details on disruption and homogenization method of tissue or cells. It is recommended to use the method of grinding sample with a pestle and mortar in the presence of liquid nitrogen to disrupt tissue samples.
(2) Too much staring materials have been used. Too much samples have more quantity of nucleic acid which can clog the Column. See "VI. Sample amount and Buffer RL volume" for details on the amount of starting material and the volume of Buffer RL.
(3) Centrifugation temperature should be at 20 - 25℃. Too low or too high temperature would affect performance of RNA Spin Column and clogged the Column.

Q2: Why is the yield of total RNA lower?
A2: (1) Insufficient disruption and homogenization. It is essential to lyse the sample completely. See details on disruption and homogenization method of tissue or cells in protocols.
(2) Too much staring material. See "VI. Sample Amount and Buffer RL Volume" for details on the amount of starting material and the volume of Buffer RL.
(3) Elute RNA insufficient. We recommend elute RNA once again. Incubation time can prolong up to 10 minutes after add RNase-Free dH_2O to the Spin Column.
(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 step as described in the protocols. Residual ethanol will occur decrease of RNA yield.

Q3: Why is purified RNA degraded?
A3: (1) Samples are not fresh. Use fresh samples as much as possible or freeze the samples in liquid nitrogen and then store at -80℃.
(2) RNase is present in the reagents and materials. Before starting the procedure, see "IV. Precautions for Preventing RNase Contamination" thoroughly.
(3) The sample is rich in RNase. It is recommended to use less starting material and increase the volume of Buffer BL for tissues and cells rich in RNase.

Q4: Why does the purified RNA have genomic DNA contamination?

A4: By the gDNA Eraser Spin Column, the most genomic DNA in the sample can be effectively removed. Generally, the purified RNA does not contain genomic DNA. If contamination with genomic DNA in RNA occurs, the reasons are mainly the following.

(1) Insufficient disruption and homogenization. It is essential to lyse the sample completely. It would result in the residue of genomic DNA. See details on disruption and homogenization method of tissue and cells in the protocols.

(2) Too much staring material used. More amount of sample can produce more quantity of nucleic acid that would exceed the loading capacity of gDNA Eraser Spin Column, and result in the residue of genomic DNA. See "VI. Sample Amount and Buffer RL Volume" for details on the amount of starting material and the volume of Buffer RL.

(3) Samples rich in genomic DNA. Genomic DNA is high in some materials. It is recommended to use less starting material and increase the volume of Buffer BL for sample rich in genomic DNA.

(4) DNase I treatment is omitted. If the downstream experiment has a strictly requirment on the purity of RNA, or a sample is rich in genomic DNA, DNase I digestion should be carried out according to the DNase digestion step in the protocols.

Q5: What is a meaning of the absorbance values of RNA?

A5 : The values of 260 nm, 320 nm, and 280 nm represent the absorbance of nucleic acid, background (turbidity of reagent), concentration of salt and protein, respectively. The value of OD260/280(R) represent the degree of contamination of organics such as protein and so on. The value should be between 1.8 and 2.2 for highly quality RNA, the ratio lower than 1.8 means that the contamination of protein is obvious and higher than 2.2 means that the RNA is degraded to single nucleic acid. Dilute the RNA using TE buffer when check the RNA absorbance values.

Q6: How to calculate the concentration of RNA?

A6: Calculate the concentration of RNA according the absorbance of RNA at different wavelength :

RNA (μg/μl) = (OD_{260}-OD_{320}) x dilution x 0.04 μg/μl

Q7: How integrity is RNA extracted?

A7: Using TaKaRa MiniBEST Universal RNA Extraction Kit, RNA longer than 200 bases can be efficiently purified.

Q8 : How quality is the genomic DNA extracted?

A8 : The genomic DNA extracted by this kit is based on an optional protocol and the yield of genomic DNA is lower than other genomic DNA extraction kit. The obtained genomic DNA is only used by PCR as template. For extraction of genomic DNA, use TaKaRa MiniBEST Universal Genomic DNA Extraction Kit 5.0 (Cat. #9765) in the case that low content of genomic DNA in samples and that requirement of higher purity of genomic DNA in downstream experiments.

NOTE : This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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