FISHER MOLECULAR BIOLOGY

Tissue Total RNA Mini Kit RE-006

-- For isolation RNA from animal cells, animal tissues, paraffin fixed tissues, bacteria, yeast, fungi and for RNA clean-up

Cat. No:	RE-006 (100 preps)	RE-006B (200 preps)
FSRB Buffer	45 ml	2 x 45 ml
Wash Buffer 1	60 ml	2 x 60 ml
Wash Buffer 2 [a](concentrate)	35 ml	2 x 35 ml
RNase-free Water	6 ml	2 x 6 ml
Filter Column	100 pcs	200 pcs
FSRB Mini Column	100 pcs	200 pcs
Collection Tube	200 pcs	400 pcs
Elution Tube	100 pcs	150 pcs
Micropestle	100 pcs	150 pcs
DNAse I	1 mg	1 mg
Preparation of Wash Buffer by adding ethanol (96 ~ 100%)		
Ethanol volume for Wash Buffer 2 **a**	140 ml	200 ml

Specifications:

Principle: mini spin column (silica matrix)

Operation time: 30 ~ 60 minutes
Binding capacity: up to 100 µg total RNA/ column
Column Applicability: centrifugation and vaccum
Minimum elution volume: 40 µl

Sample	Recommended amount of sample used		Yield (µg)
Animal cells (up to 5 x 10^6)	NIH/3T3 HeLa COS-7 LMH	1 x 10^6 cells	10 15 30 12
Animal Tissue (Mouse/rat) (up to 30 mg)	Embryo Heart Brain Kidney Liver Spleen Lung Thymus	10 mg	25 10 10 30 50 35 15 45
Bacteria	E. coli B. subtilis	1 x 10^9cells	60 40
Yeast (up to 5 x 10^7)	S. cerevisiae	1 x 10^7 cells	25

Important Notes:

1. Make sure everything is RNase-free when handling RNA.
2. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers
3. We suggest to use (ß-ME), or DTT to better to increase RNA yield.
4. **Caution: β-mercaptoethanol (β-Me) is hazardous to human health. perform the procedures involving β-Me in a chemical fume hood.**
5. Add required volume of RNase-free ethanol (96~100%) to Wash Buffer 2 when first use.
6. All centrifuge steps are done at full speed (~18,000 x g) in a microcentrifuge.
7. Prepare RNase-free DNase 1 reaction buffer (1M NaCl, 10 mM MnCl2, 20 mM Tris-HCl, pH 7.0 at 25˚C) and make the final concentration of DNase I to 0.5 U/µl.

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36 Terry Drive
Trevose, PA 19048 - USA

Protocol: Isolation of Total RNA from Animal Cells

Please Read Important Notes Before Starting Following Steps.

Additional requirment: ß-Mercaptoethanol or Dithiothreitol 1% of the buffer volume 70% RNase-free ethanol

1. Collect 1 ~ 5 ×10^6 cells by centrifuge at 300 x g for 5 min at 4 ˚C. Remove all the supernatant.

-- Note! Do not overload, too much sample will make cell lysis incompletely and lead to lower RNA yield and purity.

2. Add 350 µl of FSRB Buffer and 3.5 µl of ß-Mercaptoethanol or 3,5 µl of DTT to the cell pellet. Vortex vigorously for 1 min to resuspend the cells completely.
-- Note: If the clump is still visible after vortex, pipet sample mixture up and down to break down the clump.

3. Place a Filter Column to a Collection Tube and transfer the sample mixture to the Filter Column. Centrifuge at full speed (~ 18,000 x g) for 2 min.

4. Transfer the clarified supernatant from the Collection Tube to a new microcentrifuge tube (not provided), and measure the volume of the supernatant.
-- Note: Avoid to pipet any debris and pellet when transfering the supernatant.

5. Add 1 volume of 70 % RNase-free ethanol and mix well by vortexing.

6. Place a FSRB Mini Column to a Collection Tube and transfer the ethanol added sample mixture (including any precipitate) to the FSRB Mini Column. Centrifuge at full speed for 1 min, discard the flow-through and return the FSRB Mini Column back to the Collection Tube.

7. Optional step: DNase I digestion*Otherwise, proceed to step 8 directly.*ination, follow the steps from 7a.

7a. Add 250 µl of Wash Buffer 1 to the FSRB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the FSRB Mini Column back to the Collection Tube.

7b. Add 60 µl of RNase-free DNase 1 solution (0.5U/ul) to the membrane center of the FSRB Mini Column. Place the column on the benchtop for 15 min.

7c. Add 250 µl of Wash Buffer 1 to the FSRB Mini Column, centrifuge at ful speed for 1 min. Discard the flow-through and return the FSRB Mini Column back to the Collection Tube.

7d. After DNase 1 treatment, proceed to step 9.

8. Add 500 µl of Wash Buffer 1 to the FSRB Mini Column, centrifugeat at full speed for 1 min. Discard the flow-through and return the FSRB Mini Column back to the Collection Tube.

9. Add 750 µl of Wash Buffer 2 to the FSRB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the FSRB Mini Column back to the Collection Tube. **-- Note: Make sure that ethanol has been added into Wash Buffer 2 when first use.**

10. Repeat step 9 for one more washing.

11. Centrifuge the FSRB Mini Column at full speed for an additional 3 min to dry the FSRB Mini Column.
-- Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.

12. Place the FSRB Mini Column to a Elution Tube (provided, 1.5 ml microcentrifuge tube).

13. Add 40 ~ 100 µl of RNase-free ddH2O to the membrane center of the FSRB Mini Column. Stand the FSRB Mini Column for 1 min.
-- Important Step! For effective elution, make sure that RNase-free ddH2O is dispensed on the membrane center and is absorbed completely.
-- Important : Do not elute the RNA using RNase-free water less than suggested volume (< 40 µl). It will lower the RNA yield.

14. Centrifuge the FSRB Mini Column at full speed for 1 min to elute RNA.

15. Store RNA at -70°C.

Protocol: Isolation of Total RNA from Animal Tissues (Fresh or Frozen)

Please Read Important Notes Before Starting Following Steps.

Additional equipment: Liquid nitrogen & mortar
a rotor-stator homogenizer or a 20-G needle syringe ß-Mercaptoethanol or DTT
70% RNase-free ethanol

A-1 Weight up to 30 mg of tissue sample. Grind the sample in liquid nitrogen to a fine powder with a mortar and transfer the powder to a new microcentrifuge tube (not provided).
-- Note! Avoid thawing the sample during weighing and grinding.

A-2 Add 350 ul of FSRB Buffer and 3.5 ul of ß-Mercaptoethanol or 3,5µl of DTT. Homogenize the sample by using a rotor-stator homogenizer or by passing the sample lysate through a 20-G needle syringe 10 times. Incubate at room temperature for 5 min.**-- Important step: In order to release more RNA from the harder samples, it is recommended to homogenize the sample by using suitable homogenize equipment, for example, with a rotot-stator homogenizer.**

A-3 Follow the **Animal Cells Protocol** starting from step 3.

(Alternative)

B-1 Place up to 30 mg of tissue sample to a microcentrifuge tube. Add 350 ul of FSRB Buffer and 3.5 ul of ß-Mercaptoethanol or 3,5 µl of DTT and use provided micropestle to grind the tissue sample thoroughly.

B-2 Homogenize the sample by passing lysate through a 20-G needle syringe 10 - 20 times. Incubate at room temperature for 5 min.
-- For the tissue samples having low cell amount and hard to disrupt, it is recommended to proceed A1-A3 step above.

B-3 Follow Animal Cells Protocol starting from step 3.

Protocol: Isolation of Total RNA from Paraffin-Embedded Tissues

Please Read Important Notes Before Starting Following Steps.

Additional equipment: Xylene & ethanol (96-100%)
Liquid nitrogen & mortar
Rotor-stator homogenizer or a 20-G needle syringe
ß-Mercaptoethanol or DTT
70% RNase-free ethanol

1. Transfer up to 15 mg paraffin-embedded tissue sample to a microcentrifuge tube (not provided).
   -- **Remove the extra paraffin to minimize the size of the sample slice**.
2. Add 0.5 ml xylene, mix well and incubate at room temperature for 10 min.
3. Centrifuge at full speed for 3 min. Remove the supernatant by pipetting.
4. Add 0.25 ml xylene, mix well and incubate at room temperature for 3 min.
5. Centrifuge at full speed for 3 min. Remove the supernatant by pipetting.
6. Repeat step 4 and step 5
7. Add 0.3 ml ethanol (96- 100 %) to the deparaffined tissue, mix gently by vortexing. Incubate at room temperature for 3 min.
8. Centrifuge at full speed for 3 min. Remove the supernatant by pipetting.
9. Repeat step 7 and step 8.
10. Follow Animal tissue Protocol starting from step 1 for sample disruption then follow Animal Cells protocol starting from step 3.

Protocol: Isolation of Total RNA from Bacteria

Please Read Important Notes Before Starting Following Steps.

Additional requirment: ß-Mercaptoethanol
70% RNase-free ethanol
30 ˚C water bath or heating block 2 ml screw centrifuge tube
Lysozyme reaction solution: (10mg/ ml lysozyme; 20mM Tris-HCl, pH 8.0; 2mM EDTA; 1.2% Trition)
Acid-washed glass beads, 500 - 700 um

1. Transfer up to $1x10^9$ cells well-grown bacterial culture to a 2 ml screw centrifuge tube.
   -- **Note! Make sure the amount of total RNA harvested from sample do not excess the column's binding capacity (100 µg) when estimate the sample size. Too much sample will make cell lysis incompletely and lead to lower RNA yield and purity. If RNA amount is hard to determin on some species, using ≤ 5 x 10^8 cells as the starting sample size.**
   1. Descend the bacterial cells by centrifuge at full speed (-18,000 x g) for 2 min at 4 ˚C. Remove all the supernatant.
   2. Add 100 ul of lysozyme reaction solution. Pipet up and down to resuspend the cell pellet and incubate at 37˚C for 10 min.
   3. Add 350 ul of FSRB Buffer and 3.5 ul of ß-Mercaptoethanol or 3,5ul of DTT.
   4. Add 250 mg of acid-washed glass beads (500 - 700 nm) and vortex vigorously for 5 min to disrupt the cells.
   5. Centrifuge at full speed (-18,000 x g) for 2 min to spin down insoluble material. Transfer the supernatant to a microcentrifge tube (not provided) and measure the volume of the clear lysate.
      -- Note! Avoid pipetting any debris and pellet in the Collection Tube.
   6. Follow Animal Cells Protocol starting from step 5.

Protocol: Isolation of Total RNA from Yeast and Fungi

Please Read Important Notes Before Starting Following Steps.

Additional requirment: ß-Mercaptoethanol or DTT
70% RNase-free ethanol
Enzymatic disruption: Lyticase or zymolase
Sorbitol buffer (1 M sorbitol; 100 mM EDTA; 0.1% ß-ME)
30 ˚C water bath or heating block
Mechanical disruption: 2 ml screw centrifuge tube
Acid-washed glass beads, 500 - 700 Pm

1. Collect up to 5 x 10^7 of yeast culture by centrifuge at 5,000 x g for 10 min at 4 ˚C. Remove all the supernatant.

2A. Enzymtic disruption:
2A-1: Resuspend the cell pellet in 600 ul sorbitol buffer (1 M sorbitol; 100 mM EDTA; 0.1% ß-ME) (not provided). Add 200 U zymolase or lyticase and incubate at 30 ˚C for 30 min.
--Note! Prepare sorbitol buffer just before use.
2A-2. Centrifuge at 300 x g for 5 min to pellte the spheroplasts. Remove all the supernatant.
2A-3. Add 350 ul of FSRB Buffer and 3.5 ul of ß-Mercaptoethanol or 3,5 ul of DTT to the pellet. Vortex vigorously to disrupt the spheroplasts for 1 min. Incbuate sample mixture at room temperature for 5 min.

2B. Mechanical disruption:
2B-1. Add 350 ul of FSRB Buffer and 3.5 ul of ß-Mercaptoethanol or 3,5 ul of DTT to the pellet and vortex vigorously to resuspend the cells completely.
2B-2. Transfer the sample mixture to a 2 ml screw centrifuge tube and add 250 mg of acid-washed glass beads (500 - 700 nm) and vortex vigorously for 15 min to disrupt the cells.

3. Follow **Animal Cells Protocol** starting from step 5.

Protocol: RNA clean up

Please Read Important Notes Before Starting Following Steps.

Additional equipment: xylene & ethanol (96-100%)

1. Transfer 100 ul of RNA sample to a microcentrifuge tube (not provided).
   -- If the RNA sample is less than 100 ul, add RNase-free water to make the sample volume to 100 ul.
2. Add 300 ul of FSRB Buffer and 300 ul of RNase-free ethanol (96-100 %) and mix well by vortexing.
3. Place a FSRB Mini Column to a Collection Tube and transfer the ethanol added sample mixture to the FSRB Mini Column. Centrifuge at full speed for 1 min and discard the flow-through and return the FSRB Mini Column back to the Collection Tube.
4. DNase I digestion To eliminate genomic DNA contamination, follow the steps from 7a.

Otherwise, proceed to step 5 directly.

4a. Add 250 µl of Wash Buffer 1 to the FSRB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the FSRB Mini Column back to the Collection Tube.

4b. Add 60 µl of RNase-free DNase 1 solution (0.5U/ul, not provided) to the membrane center of the FSRB Mini Column. Place the column on the benchtop for 15 min.

4c. Add 250 µl of Wash Buffer 1 to the FSRB Mini Column, centrifuge at ful speed for 1 min. Discard the flow-through and return the FSRB Mini Column back to the Collection Tube.

5. Add 750 µl of Wash Buffer 2 to the FSRB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the FSRB Mini Column back to the Collection Tube. -- Note: Make sure that ethanol has been added into Wash Buffer 2 when first use.

6. Repeat step 5 for one more washing.

7. Centrifuge the FSRB Mini Column at full speed for an additional 3 min to dry the FSRB Mini Column. -- Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.

8. Place the FSRB Mini Column to a Elution Tube (provided, 1.5 ml microcentrifuge tube).

9. Add 40 ~ 100 µl of RNase-free ddH2O to the membrane center of the FSRB Mini Column.

Stand the FSRB Mini Column for 1 min.

-- Important Step! For effective elution, make sure that RNase-free ddH2O is dispensed on the membrane center and is absorbed completely.

-- Important : Do not elute the RNA using RNase-free water less than suggested volume (< 40 µl). It will lower the RNA yield.

10. Centrifuge the FSRB Mini Column at full speed for 1 min to elute RNA.

11. Store RNA at -70°C.

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