

Tissue Total RNA Mini Kit

RE-005

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

Cat. No:	RE-005s Mini (4 preps_sample)	RE-005 (50 preps)	RE-006 (100 preps)	RE-006B (300 preps)
FSRB Buffer	3 ml	25 ml	45 ml	130 ml
Wash Buffer 1	3 ml	30 ml	60 ml	170 ml
Wash Buffer 2 ^a (concentrate)	1.5 ml	15 ml	35 ml	50 ml x 2
RNase-free Water	0.5 ml	6 ml	6 ml	8 ml x 2
Filter Column	4 pcs	50 pcs	100 pcs	300 pcs
FSRB Mini Column	4 pcs	50 pcs	100 pcs	300 pcs
Collection Tube	8 pcs	100 pcs	200 pcs	600 pcs
Elution Tube	4 pcs	50 pcs	100 pcs	300 pcs
Micropestle	4 pcs	50 pcs	100 pcs	300 pcs
DNase I		1 mg	1 mg	1 mg
Preparation of Wash Buffer by adding ethanol (96 ~ 100%)				
Ethanol volume for Wash Buffer 2 a	6 ml	60 ml	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 vacuum

Minimum elution volume: 40 µl

Sample	Recommended amount of sample used		Yield (µg)
Animal cells (up to 5 x 10 ⁶)	NIH/3T3	1 x 10 ⁶ cells	10
	HeLa		15
	COS-7		30
	LMH		12
Animal Tissue (Mouse/rat) (up to 30 mg)	Embryo	10 mg	25
	Heart		10
	Brain		10
	Kidney		30
	Liver		50
	Spleen		35
	Lung		15
Thymus	45		
Bacteria	E. coli	1 x 10 ⁹ cells	60
	B. subtilis		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. **Caution: β-mercaptoethanol (β-Me) is hazardous to human health. perform the procedures involving β-Me in a chemical fume hood.**
4. Add required volume of RNase-free ethanol (96~100%) to Wash Buffer 2 when first use.
5. All centrifuge steps are done at full speed (~18,000 x g) in a microcentrifuge.
6. 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.

Protocol: Isolation of Total RNA from Animal Cells

Please Read Important Notes Before Starting Following Steps.

Additional requirement: β -Mercaptoethanol
70% RNase-free ethanol

1. Collect $1 \sim 5 \times 10^6$ cells by centrifuge at $300 \times g$ 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 β -Mercaptoethanol 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 ($\sim 18,000 \times g$) 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 transferring 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. To eliminate genomic DNA contamination, follow the steps from 7a.
Otherwise, proceed to step 8 directly.
 - 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/ μl) 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 full 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, centrifuge 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 \sim 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 ($< 40 \mu\text{l}$). 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
 β -Mercaptoethanol
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 μl of FSRB Buffer and 3.5 μl of β -Mercaptoethanol. 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 μl of FSRB Buffer and 3.5 μl of β -Mercaptoethanol 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
 β -Mercaptoethanol
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 requirement: β -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% Triton)
Acid-washed glass beads, 500 - 700 μ m

1. Transfer up to 1×10^9 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 exceed the column's binding capacity (100 μ g) 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 determine on some species, using $\leq 5 \times 10^8$ cells as the starting sample size.**
 1. Descend the bacterial cells by centrifuge at full speed ($-18,000 \times g$) for 2 min at 4 °C. Remove all the supernatant.
 2. Add 100 μ l of lysozyme reaction solution. Pipet up and down to resuspend the cell pellet and incubate at 37°C for 10 min.
 3. Add 350 μ l of FSRB Buffer and 3.5 μ l of β -Mercaptoethanol.
 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 ($-18,000 \times g$) for 2 min to spin down insoluble material. Transfer the supernatant to a microcentrifuge 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 requirement: β -Mercaptoethanol
70% RNase-free ethanol
Enzymatic disruption: Lyticase or zymolase
Sorbitol buffer (1 M sorbitol; 100 mM EDTA; 0.1% β -ME)
30 °C water bath or heating block
Mechanical disruption: 2 ml screw centrifuge tube
Acid-washed glass beads, 500 - 700 μ m

1. Collect up to 5×10^7 of yeast culture by centrifuge at $5,000 \times g$ for 10 min at 4 °C. Remove all the supernatant.
- 2A. Enzymtic disruption:
 - 2A-1: Resuspend the cell pellet in 600 μ l sorbitol buffer (1 M sorbitol; 100 mM EDTA; 0.1% β -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 $300 \times g$ for 5 min to pellet the spheroplasts. Remove all the supernatant.
 - 2A-3. Add 350 μ l of FSRB Buffer and 3.5 μ l of β -Mercaptoethanol to the pellet. Vortex vigorously to disrupt the spheroplasts for 1 min. Incubate sample mixture at room temperature for 5 min.
- 2B. Mechanical disruption:
 - 2B-1. Add 350 μ l of FSRB Buffer and 3.5 μ l of β -Mercaptoethanol 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 μ l of RNA sample to a microcentrifuge tube (not provided).
-- If the RNA sample is less than 100 μ l, add RNase-free water to make the sample volume to 100 μ l.
2. Add 300 μ l of FSRB Buffer and 300 μ l 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 μ l 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 μ l of RNase-free DNase 1 solution (0.5U/ μ l, not provided) to the membrane center of the FSRB Mini Column. Place the column on the benchtop for 15 min.
 - 4c. Add 250 μ l 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.
5. Add 750 μ l 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 μ l 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 μ l). 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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