FavorPrep™ Tissue Total RNA Extraction Maxi Kit

-For isolation RNA from animal tissue, animal cells, bacterial cells and yeast cells

For Research Use Only

Kit Contents:

Cat. No. (preps)	FATR1050 (2 preps)	FATR1051 (10 preps)	FATR1052 (25 preps)
FARB Buffer	30 ml	150 ml	180 ml × 2
Wash Buffer 1	30 ml	135 ml	160 ml × 2
Wash Buffer 2 ■ (Concentrate)	12 ml	54 ml	45 ml × 3
RNase-Free Water	5 ml	15 ml	30 ml
Filter Columns	2 pcs	10 pcs	25 pcs
RNA Maxi Columns	2 pcs	10 pcs	25 pcs
50 ml centrifuge tubes	4 pcs	20 pcs	50 pcs
User manual	1	1	1

■ Add RNase-free ethanol (96~100%) to Wash Buffer 2 for the first open.

	FATR1050	FATR1051	FATR1052
Ethanol volume for Wash Buffer 2	48 ml	216 ml	180 ml

Specification:

Principle: maxi spin column (silica matrix)
Operation time: 60 mins
Binding capacity: up to 2000 µg total RNA/column
Column applicability: centrifugation and vacuum
Minimum elution volume: 500 µl

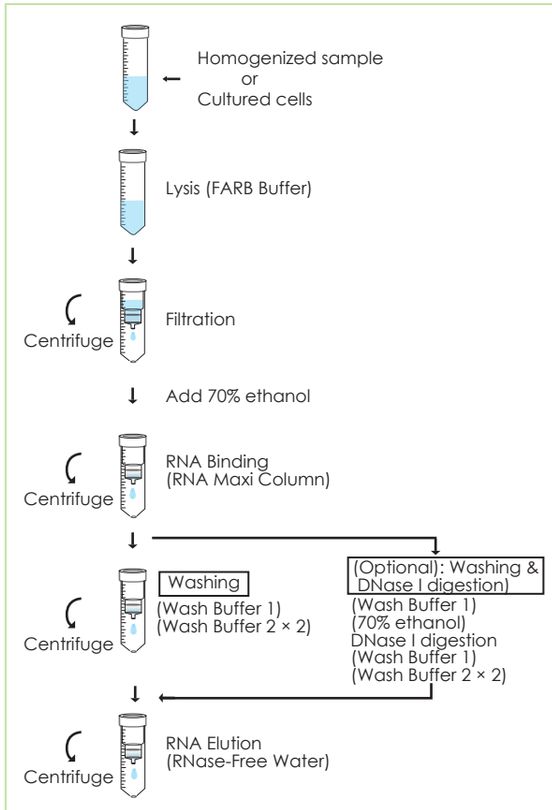
Recommend sample size

Animal tissue: ≤650 mg
Animal cells: ≤1.5×10^8
Bacterial cells: ≤3×10^{10}
Yeast cells: ≤1×10^9

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: ß-mercaptoethanol (ß-Me) is hazardous to human health. Perform the procedures involving ß-Me in a chemical fume hood.**
4. Add required volume of RNase-free ethanol (96~100%) to Wash Buffer 2 at the first use.
5. All centrifugation steps are done by swing-bucke rotor to make RNA bind symmetrically and at speed 4,500~6,000 xg.
6. (Optional) For each reaction, prepare 1 ml of RNase-free DNase I solution (0.25 U/µl). Prepare a 10× DNase I reaction buffer containing 1 M NaCl, 10 mM $MnCl_2$ or $MgCl_2$, and 20 mM Tris-HCl (pH 7.0 at 25°C). Dilute this buffer to a 1× working concentration before use. Use the 1× buffer to dilute the DNase I enzyme to a final concentration of 0.25 U/µl. Alternatively, use the ready-to-use **FavorPrep™ DNase I Solution (Cat. No. FADI2093)** to simplify preparation.

Brief procedure:



Protocol: Animal Tissues

Please Read Important Notes Before Starting Following Steps.

Additional requirement: 50 ml centrifuge tube
Liquid nitrogen & mortar
Rotor-stator homogenizer
ß-Mercaptoethanol
RNase-free 70% ethanol
Swing-bucket centrifugator for 50 ml tube
55°C oven

1 Weight ≤650 mg of animal tissue. Grind the sample in liquid nitrogen to a fine powder with a mortar and transfer the powder to a 50 ml centrifuge tube (not provided).
-Note: Avoid thawing the sample during weighing and grinding.
2. Add 14 ml of FARB Buffer and 0.14 ml of ß-Mercaptoethanol. Homogenize the sample by using a rotor-stator homogenizer. Incubate at room temperature for 5 mins.
-Important step: To release more RNA from the harder samples, it is recommended to homogenize the sample by using suitable homogenize equipment, for example, with a rotor-stator homogenizer.
3. Place a Filter Column to a 50 ml centrifuge tube (Provided) and transfer the sample mixture to the Filter Column. Close the cap and centrifuge at 4,500 xg for 5 mins.
4. Transfer 12 ml of the clarified supernatant from the 50 ml centrifuge tube to a new 50 ml centrifuge tube. (not provided)
-Note: Avoid to pipette any floating turbid debris when transferring the supernatant.
5. Add 12 ml of RNase-free 70% ethanol and mix well by vortexing for 5 secs.
6. Place a RNA Maxi Column to a new 50 ml centrifuge tube (provided) and transfer up to 12 ml of the ethanol added mixture to the RNA Maxi Column. Close the cap and centrifuge at 4,500 xg for 2 mins. Discard the flow-through and return the RNA Maxi Column back to the 50 ml centrifuge tube.
7. Repeat step 6 for the rest 12 ml of the sample mixture

8. Optional step: DNase I digestion To eliminate genomic DNA contamination, follow the steps from 8a. Otherwise, proceed to step 9 directly.
8a. Add 6 ml of Wash Buffer 1 to the RNA Maxi Column. Close the cap and centrifuge at 4,500 xg for 2 mins. Discard the flow-through and return the RNA Maxi Column back to the 50 ml centrifuge tube.
8b. Add 12 ml of RNase-free 70% ethanol to the RNA Maxi Column. Close the cap and centrifuge at 4,500 xg for 2 mins. Discard the flow-through and return the RNA Maxi Column back to the 50 ml centrifuge tube.
8c. Add 1 ml of RNase-free DNase I solution (0.25 U/µl, not provided) to the membrane center of the RNA Maxi Column. Place the column on the benchtop for 15 mins.
8d. Add 6 ml of Wash Buffer 1 to the RNA Maxi Column. Close the cap and centrifuge at 4,500 xg for 2 mins. Discard the flow-through and return the RNA Maxi Column back to the 50 ml centrifuge tube.
8e. After DNase I treatment, proceed to step 10.

9. Add 12 ml of Wash Buffer 1 to the RNA Maxi Column. Close the cap and centrifuge at 4,500 xg for 2 mins. Discard the flow-through and return the RNA Maxi Column back to the 50 ml centrifuge tube.
10. Add 12 ml of Wash Buffer 2 to the RNA Maxi Column. Close the cap and centrifuge at 4,500 xg for 2 mins. Discard the flow-through and return the RNA Maxi Column back to the 50 ml centrifuge tube.
-Note: Make sure that ethanol has been added into Wash Buffer 2 at the first use.
11. Repeat step 10 for one more washing. Add 12 ml of Wash Buffer 2 to the RNA Maxi Column. Close the tube cap and centrifuge at 4,500~6,000 xg for 10 mins. Discard the flow-through.
12. Place the RNA Maxi Column to a new 50 ml centrifuge tube (not provided).
13. Incubate the RNA Maxi Column at 55°C oven for 10 mins.
-Note: The oven incubation should be 10 mins to remove the wash buffer from membranes completely.
14. Add 500 µl of RNase-Free Water to the membrane center of the RNA Maxi Column. Stand the RNA Maxi Column for 2 mins at room temperature.
-Important Step! For effective elution, make sure that RNase-Free Water is dispensed on the membrane center and is absorbed completely.
15. Close the tube cap and centrifuge at 4,500~6,000 xg for 2 mins to elute RNA.
16. Use the purified RNA in downstream application or store RNA at -70°C.

Protocol: Animal Cells

Please Read Important Notes Before Starting Following Steps.

Additional requirement: 50 ml centrifuge tube
ß-Mercaptoethanol
RNase-free 70% ethanol
Swing-bucket centrifugator for 50 ml tube
55°C oven

1. Collect ≤1.5×10^8 cells in a 50 ml centrifuge tube (not provided) by centrifuge at 300 xg for 5 mins 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 14 ml FARB Buffer and 0.14 ml of ß-Mercaptoethanol to the cell pellet. Vortex vigorously for 1 min to resuspend the cells completely and incubate the sample at room temperature for 5 mins.
   -Note: If the clump is still visible after vortex, pipette sample mixture up and down to break down the clump.
3. Place a Filter Column to a 50 ml centrifuge tube (Provided) and transfer the sample mixture to the Filter Column. Close the cap and centrifuge at 4,500 xg for 5 mins.
4. Transfer 12 ml of the clarified supernatant from the 50 ml centrifuge tube to a new 50 ml centrifuge tube. (not provided)
   -Note: Avoid to pipette any floating turbid debris when transferring the supernatant.
5. Add 12 ml of RNase-free 70% ethanol and mix well by vortexing for 5 secs.
6. Place a RNA Maxi Column to a new 50 ml centrifuge tube (provided) and transfer up to 12 ml of the ethanol added mixture to the RNA Maxi Column. Close the cap and centrifuge at 4,500 xg for 2 mins. Discard the flow-through and return the RNA Maxi Column back to the 50 ml centrifuge tube.
7. Repeat step 6 for the rest 12 ml of the sample mixture.

8. Optional step: DNase I digestion To eliminate genomic DNA contamination, follow the steps from 8a. Otherwise, proceed to step 9 directly.
   8a. Add 6 ml of Wash Buffer 1 to the RNA Maxi Column. Close the cap and centrifuge at 4,500 xg for 2 mins. Discard the flow-through and return the RNA Maxi Column back to the 50 ml centrifuge tube.
   8b. Add 12 ml of RNase-free 70% ethanol to the RNA Maxi Column. Close the cap and centrifuge at 4,500 xg for 2 mins. Discard the flow-through and return the RNA Maxi Column back to the 50 ml centrifuge tube.
   8c. Add 1 ml of RNase-free DNase I solution (0.25 U/µl, not provided) to the membrane center of the RNA Maxi Column. Place the column on the benchtop for 15 mins.
   8d. Add 6 ml of Wash Buffer 1 to the RNA Maxi Column. Close the cap and centrifuge at 4,500 xg for 2 mins. Discard the flow-through and return the RNA Maxi Column back to the 50 ml centrifuge tube.
   8e. After DNase I treatment, proceed to step 10.

9. Add 12 ml of Wash Buffer 1 to the RNA Maxi Column. Close the cap and centrifuge at 4,500 xg for 2 mins. Discard the flow-through and return the RNA Maxi Column back to the 50 ml centrifuge tube.
10. Add 12 ml of Wash Buffer 2 to the RNA Maxi Column. Close the cap and centrifuge at 4,500 xg for 2 mins. Discard the flow-through and return the RNA Maxi Column back to the 50 ml centrifuge tube.
    -Note: Make sure that ethanol has been added into Wash Buffer 2 at the first use.
11. Repeat step 10 for one more washing. Add 12 ml of Wash Buffer 2 to the RNA Maxi Column. Close the tube cap and centrifuge at 4,500~6,000 xg for 10 mins. Discard the flow-through.
12. Place the RNA Maxi Column to a new 50 ml centrifuge tube (not provided).
13. Incubate the RNA Maxi Column at 55°C oven for 10 mins.
    -Note: The oven incubation should be 10 mins to remove the wash buffer from membranes completely.
14. Add 500 µl of RNase-Free Water to the membrane center of the RNA Maxi Column. Stand the RNA Maxi Column for 2 mins at room temperature.
    -Important Step! For effective elution, make sure that RNase-Free Water is dispensed on the membrane center and is absorbed completely.
15. Close the tube cap and centrifuge at 4,500~6,000 xg for 2 mins to elute RNA.
16. Use the purified RNA in downstream application or store RNA at -70°C.

Protocol: Bacterial cells

Please Read Important Notes Before Starting Following Steps.

Additional requirment: 50 ml centrifuge tube
ß-Mercaptoethanol
RNase-free 70% ethanol
37°C water bath or heating block
Lysozyme reaction solution: (10 mg/ml lysozyme; 20 mM Tris-HCl, pH 8.0; 2 mM EDTA; 1.2% Trition)
Acid-washed glass beads, 500~700 µm
Swing-bucket centrifugator for 50 ml tube
55°C oven

1. Transfer ≤3×10^{10} cells well-grown bacterial culture to a 50 ml centrifuge tube.
   -Note: Make sure the amount of total RNA harvested from sample do not excess the column's binding capacity (2000 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 ≤1.5×10^{10} cells as the starting sample size.
2. Descend the bacterial cells by centrifuge at speed (4,500~6,000 xg) for 5 mins at 4°C. Remove all the supernatant.
3. Add 1 ml of lysozyme reaction solution. Pipette up and down to resuspend the cell pellet and incubate at 37°C for 10 mins.
4. Add 14 ml FARB Buffer and 0.14 ml of ß-Mercaptoethanol.
5. Add 500 mg of acid-washed glass beads (500~700 µm) and vortex vigorously for 5 mins to disrupt the cells.
6. Incubate the sample at room temperature for 5 mins.
7. Follow Animal Cells Protocol starting from step 3.

Protocol: Yeast

Please Read Important Notes Before Starting Following Steps.

Additional requirment: 50 ml centrifuge tube
ß-Mercaptoethanol
RNase-free 70% ethanol
Swing-bucket centrifugator for 50 ml tube
55°C oven
Enzymatic disruption: Lyticase or zymolyase
Sorbitol buffer (1 M sorbitol; 100 mM EDTA; 0.1% ß-ME)
30°C water bath or heating block

1. Collect ≤1×10^9 of yeast culture by centrifuge at 4,500 xg for 5 mins at 4°C. Remove all the supernatant.
2. Resuspend the cell pellet in 2.4 ml sorbitol buffer (1 M sorbitol; 100 mM EDTA; 0.1% ß-ME) (not provided).
   -Note: Prepare sorbitol buffer just before use.
3. Add 800 U lyticase or zymolyase and incubate at 30°C for 30 mins.
4. Centrifuge at 300 xg for 5 mins to pellte the spheroplasts. Remove all the supernatant.
5. Add 14 ml FARB Buffer and 0.14 ml of ß-Mercaptoethanol to the pellet. Vortex vigorously to disrupt the spheroplasts for 1 min. Incbuate sample mixture at room temperature for 5 mins.
6. Follow Animal Cells Protocol starting from step 3.