FavorPrep™ Tissue/Cultured Cells Total RNA Maxi Kit

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

For Research Use Only

Kit Contents:

Cat. No. / preps	FATRK 003-S (2 preps)	FATRK 003 (10 preps)	FATRK 003-1 (20 preps)
FARB Buffer	30 ml	150 ml	300 ml
Wash Buffer 1	30 ml	150 ml	300 ml
Wash Buffer 2 ■ (concentrate)	12 ml	50 ml	50 ml x 2
RNase-free ddH2O	5 ml	15 ml	30 ml
Filter Columns	2 pcs	10 pcs	20 pcs
RNA Maxi Columns	2 pcs	10 pcs	20 pcs
50 ml centrifuge tubes	4 pcs	20 pcs	40 pcs
User manual	1	1	1

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

	FATRK 000-S	FATRK 003	FATRK 003-1
* Ethanol volume for Wash Buffer 2	48 ml	200 ml	200 ml

Specification:

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

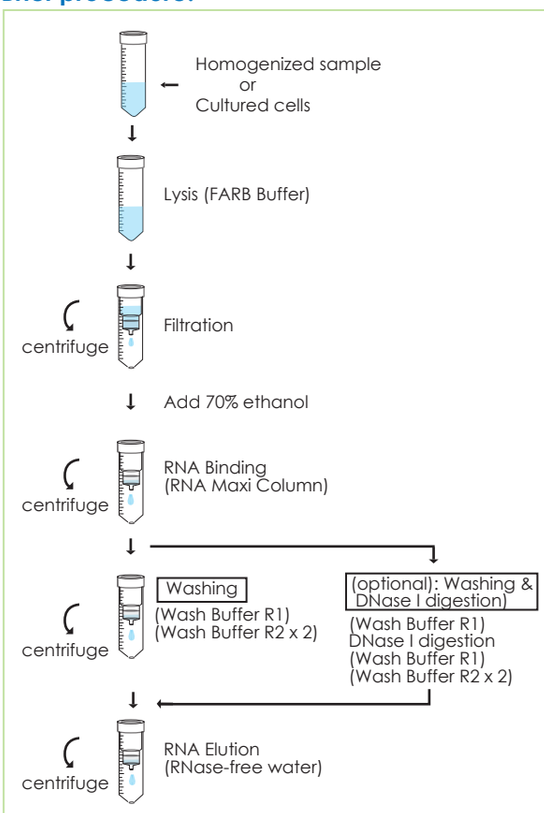
Recommend sample size

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 when first use.
5. All centrifugation steps are done by swing-bucket rotor to make RNA bind symmetrically and at speed 4,500 ~ 6,000 x g.
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.

Brief procedure:



Protocol: Animal Tissues

Please Read Important Notes Before Starting Following Steps.

Additional equipment: 50 ml centrifuge tube
liquid nitrogen & mortar
a rotor-stator homogenizer
ß-Mercaptoethanol
70% RNase-free 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 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.
3. Place a Filter Column to a 50 ml centriguge tube (Provided) and transfer the sample mixture to the Filter Column. Close the cap and centrifuge at 4,500 x g for 5 min. Discard the flow-through and place the column back to the 50 ml centrifuge tube.
-- Note: The centrifuge rotor of 50 ml tube should be swig-bucket type to make plasmid bind symmetrically to the membranes.
4. Transfer 12 ml of the clarified supernatant from a new 50 ml centrifuge tube to a new 50 ml centrifuge tube. (not provided)
-- Note: Avoid to pipet any turbid debris floating above the pellet when transferring the supernatant.
5. Add 12 ml of 70 % RNase-free ethanol and mix well by vortexing for 5 seconds.
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 x g for 2 min. 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 5 ml of Wash Buffer 1 to the RNA Maxi Column. Close the cap and centrifuge at 4,500 x g for 2 min. Discard the flow-through and return the RNA Maxi Column back to the 50 ml centrifuge tube.
8b. Add 500 µl of RNase-free DNase 1 solution (0.5U/ul, not provided) to the membrane center of the RNA Maxi Column. Place the column on the benchtop for 15 min.
8c. Add 5 ml of Wash Buffer 1 to the RNA Micro Column. Close the cap and centrifuge at 4,500 x g for 2 min. Discard the flow-through and return the RNA Maxi Column back to the 50 ml centrifuge tube.
8d. After DNase 1 treatment, proceed to step 10.

9. Add 10 ml of Wash Buffer 1 to the RNA Maxi Column. Close the cap and centrifuge at 4,500 x g for 2 min. 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 x g for 2 min. 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 when 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 x g for 10 min. 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 min.
Note: The oven incubation should be 10 min to remove the wash Buffer from membranes completely.
14. Add 500 µl of RNase-free ddH2O to the membrane center of the RNA Micro Column. Stand the RNA Maxi Column for 2 min at room temperature.
--**Important Step!** For effective elution, make sure that RNase-free ddH2O is dispensed on the membrane center and is absorbed completely.
15. Close the tube cap and centrifuge at 4,500 ~6,000 x g for 2 min to elute RNA.
16. Use the purified RNA in downstream application or store RNA at -70C.

Protocol: Animal Cells

Please Read Important Notes Before Starting Following Steps.

Additional equipment: 50 ml centrifuge tube
ß-Mercaptoethanol
70% RNase-free 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 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 14 ml 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 min.
   -- 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 50 ml centriguge tube (Provided) and transfer the sample mixture to the Filter Column. Close the cap and centrifuge at 4,500 x g for 5 min. Discard the flow-through and place the column back to the 50 ml centrifuge tube.
   -- Note: The centrifuge rotor of 50 ml tube should be swig-bucket type to make plasmid bind symmetrically to the membranes.
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 pipet any turbid debris floating above the pellet when transferring the supernatant.
5. Add 12 ml of 70 % RNase-free ethanol and mix well by vortexing for 5 seconds.
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 x g for 2 min. 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 5 ml of Wash Buffer 1 to the RNA Maxi Column. Close the cap and centrifuge at 4,500 x g for 2 min. Discard the flow-through and return the RNA Maxi Column back to the 50 ml centrifuge tube.
   8b. Add 500 µl of RNase-free DNase 1 solution (0.5U/ul, not provided) to the membrane center of the RNA Maxi Column. Place the column on the benchtop for 15 min.
   8c. Add 5 ml of Wash Buffer 1 to the RNA Micro Column. Close the cap and centrifuge at 4,500 x g for 2 min. Discard the flow-through and return the RNA Maxi Column back to the 50 ml centrifuge tube.
   8d. After DNase 1 treatment, proceed to step 10.

9. Add 10 ml of Wash Buffer 1 to the RNA Maxi Column. Close the cap and centrifuge at 4,500 x g for 2 min. 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 x g for 2 min. 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 when 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 x g for 10 min. 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 min.
    Note: The oven incubation should be 10 min to remove the wash Buffer from membranes completely.
14. Add 500 µl of RNase-free ddH_2O to the membrane center of the RNA Micro Column. Stand the RNA Maxi Column for 2 min at room temperature.
    --**Important Step!** For effective elution, make sure that RNase-free ddH_2O is dispensed on the membrane center and is absorbed completely.
15. Close the tube cap and centrifuge at 4,500 ~ 6,000 x g for 2 min to elute RNA.
16. Use the purified RNA in downstream application or store RNA at -70C.

Protocol: Bacterial cells

Please Read Important Notes Before Starting Following Steps.

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

1. Transfer ≤ 3 x10^{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 (35 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 x 10^{10} cells as the starting sample size.
2. Descend the bacterial cells by centrifuge at speed (4,500 ` 6,000 x g) for 5 min at 4 °C. Remove all the supernatant.
3. Add 1 ml of lysozyme reaction solution. Pipet up and down to resuspend the cell pellet and incubate at 37°C for 10 min.
4. Add 14 ml and 0.14 ml of ß-Mercaptoethanol.
5. Add 500 mg of acid-washed glass beads (500 ~ 700 nm) and vortex vigorously for 5 min to disrupt the cells.
6. Incubate the sample at room temperature for 5 min.
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
70 % RNase-free ethanol
swing-bucket centrifugator for 50 ml tube
55 °C oven
Enzymatic disruption: Lyticase or zymolase
Sorbitol buffer (1 M sorbitol; 100 mM EDTA; 0.1% ß-ME)
30 °C water bath or heating block

1. Collect ≤ 1 x 10^9 of yeast culture by centrifuge at 4,500 x g for 5 min 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).
3. Add 800 U lyticase or zymolase and incubate at 30 °C for 30 min.
   --Note! Prepare sorbitol buffer just before use.
4. Centrifuge at 300 x g for 5 min 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 min.
6. Follow Animal Cells Protocol starting from step 3.