FavorPrep™ Total RNA Plus Mini Kit

-- For isolation total RNA from animal cells and tissues.
-- Efficient removal of genomic DNA by using gDNA Removal Column, without the need for DNase I digestion.

For Research Use Only

Kit Contents:

Cat. No:	FATRK-P-004 (4 preps)	FATRK-P-050 (50 preps)	FATRK-P-100 (100 preps)
Lysis Buffer RXB	1.6 ml	20 ml	40 ml
Wash Buffer (concentrate) *	1.5 ml	15 ml	35 ml
RNase-free Water	0.5 ml	6 ml	6 ml
gDNA Removal Column (green)	4 pcs	50 pcs	100 pcs
RNA Mini Column	4 pcs	50 pcs	100 pcs
Collection Tube	12 pcs	150 pcs	300 pcs
Elution Tube	4 pcs	50 pcs	100 pcs
User Manual	1	1	1
Preparation of Wash Buffer by adding ethanol (96 ~ 100%)			
* Ethanol volume for Wash Buffer	6 ml	60 ml	140 ml

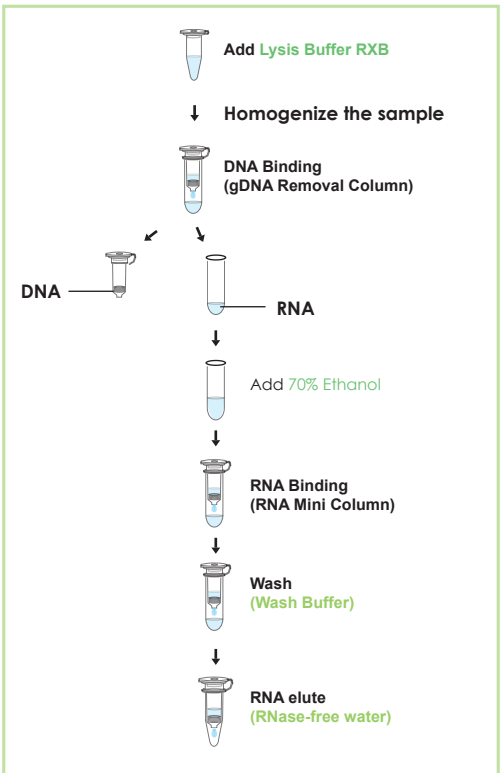
Specification:

Principle: spin column (silica membrane)
Sample size : animal cells up to 10^7
Tissue up to 30 mg
Elution volume: 30 ~ 50 µl

Important Notes:

1. Make sure the starting sample amount is under the limit.
2. Make sure everything is RNase-free when handling RNA.
3. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling hese buffers.
4. Add required volume of RNase-free ethanol (96~100%) to Wash Buffer when first use.

Brief Procedure:



Protocol: Isolation of Total RNA from Animal Cells

Please Read Important Notes Before Starting Following Steps.

Additional requirment: ß-Mercaptoethanol
70% RNase-free ethanol
rotor-stator homogenizer or 20-G needle syringe

1. Collect up to 10^7 cells by centrifuge at 300 x g for 5 min at 4 °C. Remove all the supernatant.
   Add 350 µl of Lysis Buffer RXB and 3.5 µl of ß-Mercaptoethanol to the cell pellet. Vortex vigorously for 1 min to resuspend the cells completely.
   -- Note! Do not overload, too much sample will make cell lysis incompletely and lead to lower RNA yield and purity.
2. Homogenize the sample by using a rotor-stator homogenizer or by passing the sample lysate through a 20-G needle syringe 10 times.
   -- Important step: In order to release more RNA from samples, it is recommended to homogenize the sample by using suitable homogenize equipment, for example, with a rotot-stator homogenizer.
3. Incubate at room temperature for 5 min.
4. Place a gDNA Removal Column to a Collection Tube and transfer the sample lysate to the gDNA Removal Column.
5. Centrifuge at full speed (~ 18,000 x g) for 1 min. After centrifugation, do not discard the flow-though inside the Collection tube.
6. Transfer the supernatant of flow-through from step 5 to a 1.5 ml tube (not provided). Measure the volume of the supernatant.
7. Add 1 volume of 70% ethanol and mix well by plus-vortexing.
   Example: add 330 µl of 70 % ethanol to 330 µl of supernatant from step 6.
8. Place a RNA Mini Column in a Collection Tube and transfer the sample mixture to the RNA Mini Column.
9. Centrifuge at full speed (~ 18,000 x g) for 1 min. Discard the flow-through and place the RNA Mini Column back to Collection Tube.
10. Add 500 µl of Wash Buffer to the RNA Mini Column. Centrifuge at full speed (~ 18,000 x g) for 1 min. Discard the flow-through and return the RNA Mini Column back to the Collection Tube.
    -- Note: Make sure that ethanol has been added to Wash Buffer when first use.
11. Repeat step 10 for one more washing.
12. Centrifuge the RNA Mini Column at full speed (~ 18,000 x g) for an additional 3 min to dry the RNA Mini Column.
    -- Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.
13. Place the RNA Mini Column to a Elution Tube (provided, 1.5 ml microcentrifuge tube).
14. Add 30 ~ 50 µl of RNase-free ddH_2O to the membrane center of the RNA Mini Column. Stand the RNA Mini Column at room temperature for 1 min.
    -- Important Step! For effective elution, make sure that RNase-free ddH_2O is dispensed on the membrane center and is absorbed completely.
    -- Important : Do not elute the RNA using RNase-free water less than suggested volume. It will lower the RNA yield.
15. Centrifuge the RNA Mini Column at full speed (~ 18,000 x g) for 1 min to elute RNA. Store RNA at -80 °C.

Protocol: Isolation of Total RNA from Animal Tissues

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
70% RNase-free ethanol

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). Add 350 µl of Lysis Buffer RX and 3.5 µl of ß-Mercaptoethanol.
2. Homogenize the sample by using a rotor-stator homogenizer or by passing the sample lysate through a 20-G needle syringe 10 times.
   -- Note! Avoid thawing the sample during weighing and grinding.
   -- Note! Do not overload, too much sample will make cell lysis incompletely and lead to lower RNA yield and purity.
3. Follow the Animal Cells Protocol starting from step 3.