FAVORGEN BIOTECH CORP.

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

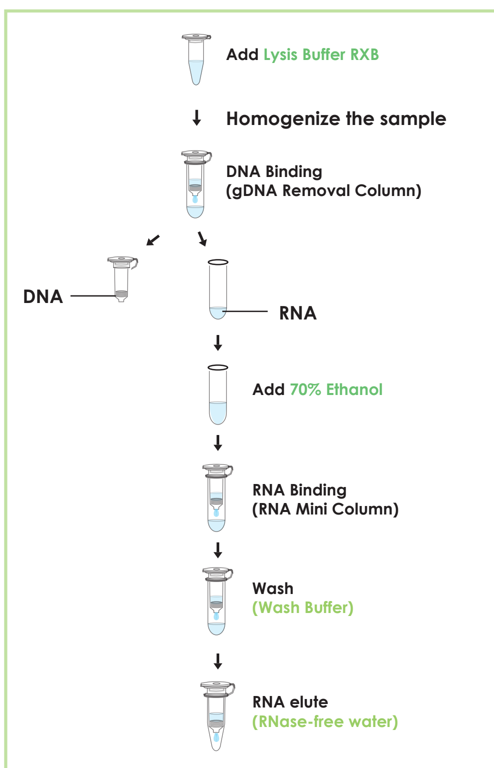
Specification:

Principle: spin column (silica membrane)
Sample size : up to 1 x 10^7 animal cells
up to 30 mg Tissue
Elution volume: 30 ~ 50 µl

Important Notes:

1. Do not exceed the maximum recommended sample size given at the beginning of each protocol.
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 these 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 requirement: ß-Mercaptoethanol
70% RNase-free ethanol
rotor-stator homogenizer or 20-G needle syringe

1. Collect up to 1 ×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 rotor-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.
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 an 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 requirement: 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 RXB 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.