



FavorPrep™ Whole Blood RNA Mini Kit

-For isolation of total RNA from whole blood

For Research Use Only

Kit Contents:

Cat. No.:	FAWBR004 (4 preps)	FAWBR050 (50 preps)	FAWBR100 (100 preps)
Lysis Buffer CX	1.5 ml	20 ml	40 ml
Wash Buffer R1 (Concentrate)	1 ml ^(a)	13 ml ^(b)	26 ml ^(c)
Wash Buffer R2 (Concentrate)	1.5 ml ^(d)	15 ml ^(e)	30 ml ^(f)
RNase-free Water	0.5 ml	6 ml	6 ml
Proteinase K (Liquid)	100 µl × 2	1050 µl × 2	1050 µl × 4
RNA Binding Column	4 pcs	50 pcs	100 pcs
Collection Tube	8 pcs	100 pcs	200 pcs
Elution Tube	4 pcs	50 pcs	100 pcs
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■ Preparation of Wash Buffer R1 by adding (a) 1.3 ml, (b) 17 ml, (c) 34 ml of ethanol (96~100%).
◆ Preparation of Wash Buffer R2 by adding (d) 6 ml, (e) 60 ml, (f) 120 ml of ethanol (96~100%).

Storage:

All component of FavorPrep™ Whole Blood RNA Mini Kit should be stored at room temperature (15~25°C).

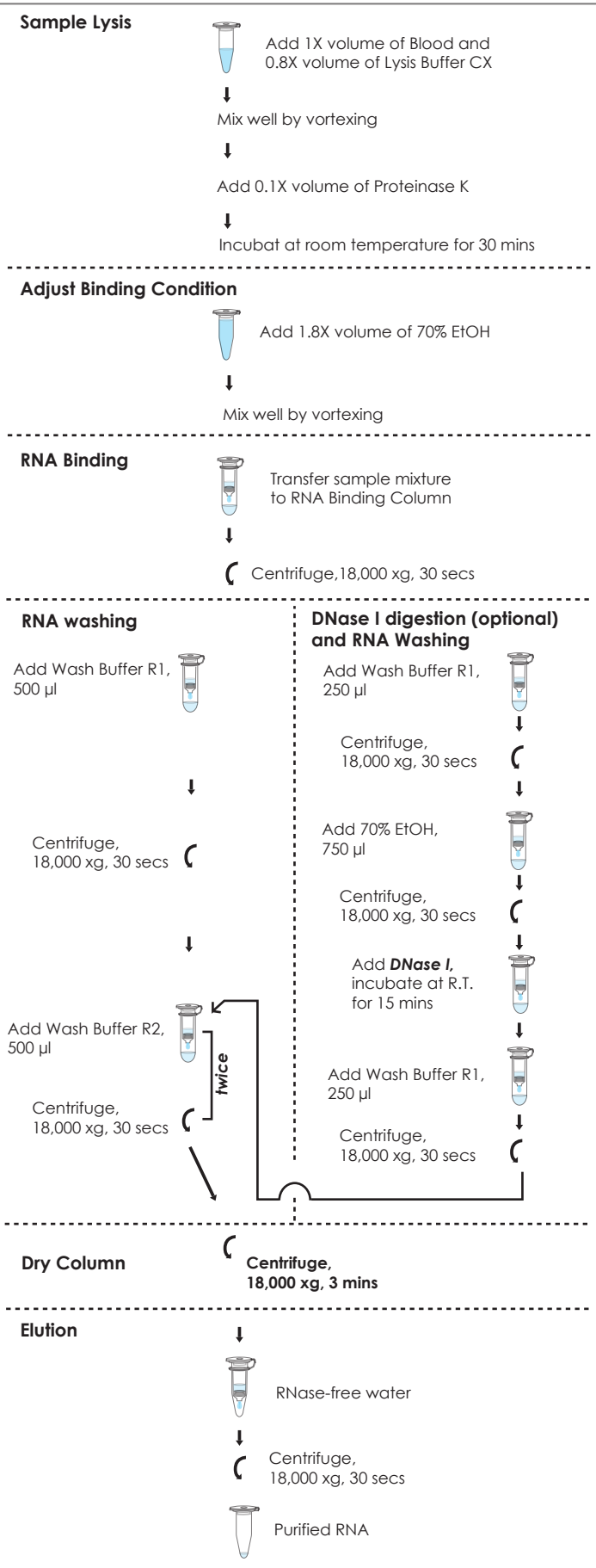
Quality Control:

The quality of FavorPrep™ Whole Blood RNA Mini Kit is tested on a lot-to-lot basis. 200 µl of whole blood were processed according to the Protocol “Isolation of total RNA from Whole Blood”. The yield of RNA should be at least reach to 6 µg determined by the absorbance at 260 nm (A260) using spectrophotometer. RNA purity was determined by A260/A280 ratio at pH 7.0 and the ratio should be between 1.9~2.0. The integrity of isolated RNA was check by RIN ≥7 on capillary electrophoresis.

Specification:

1. Format: mini spin column (RNA Binding Column)
2. Principle: silica-membrane technology/chaotropic salt binding
3. Sample size: 200~400 µl of whole blood
4. Size of isolated RNA: >200 nucleotides
5. Typical RNA yield: 5~7 µg of whole blood
6. Operation time: ≤50 mins
7. Binding capacity: ≤100 µg RNA/Column
8. Column applicability: centrifugation and vacuum
9. Minimum elution volume: 30 µl

Procedure Overview:



Protocol: Isolation of Total RNA from Whole Blood

Please Read Important Notes and Safety information Before Starting Following Steps.

1. Sample Lysis

- 1-1. Transfer ● 200 µl or ▲ 400 µl of whole blood sample to a microcentrifuge tube (not provided).
-If the sample volume is less than 200 µl or 400 µl, add the appropriate volume of PBS.
- 1-2. Add 0.8X volume of Lysis Buffer CX (● 160 µl or ▲ 320 µl) to the sample. **Mix thoroughly by pulse-vortexing for 10 secs.**
- 1-3. Briefly spin the tube to remove drops inside of the lid.
- 1-4. Add 0.1X volume of Proteinase K (● 20 µl or ▲ 40 µl) to the sample. **Mix thoroughly by pulse-vortexing.**
-Note: Do not add Proteinase K directly to Lysis Buffer CX.
- 1-5. Incubate at room temperature for 30 mins.
During incubation, vortex the sample every 10 mins.
- 1-6. Briefly spin the tube to remove drops inside of the lid.

2. Adjust Binding Condition

- 2-1. Add 1.8X volume of 70% ethanol (● 360 µl or ▲ 720 µl) to the sample mixture. **Mix thoroughly by pulse-vortexing for 10 secs.**
- 2-2. Briefly spin the tube to remove drops inside of the lid.

3. RNA Binding

- 3-1. Place an RNA Binding Column to a Collection Tube.
- 3-2. Transfer the sample mixture carefully to the RNA Binding Column. Centrifuge at 6,000 xg for 30 secs, **then place the RNA Binding Column to a new Collection Tube.**

4. DNase I digestion (optional) & RNA Washing

Steps 4-1-a to 4-1-e are for elimination of genomic DNA contamination. Otherwise, proceed to step 4-2 directly.

- 4-1-a. Add 250 µl of Wash Buffer R1 to the RNA Binding Column. Centrifuge at 18,000 xg for 30 secs. Discard the flow-through and return the RNA Binding Column back to the Collection Tube.
-Note: Make sure that ethanol has been added into Wash Buffer R1 at the first use.
- 4-1-b. Add 750 µl of 70% ethanol to the RNA Binding Column. Centrifuge at 18,000 xg for 30 secs. Discard the flow-through and return the RNA Binding Column back to the Collection Tube.
- 4-1-c. Add 60 µl of RNase-free DNase I solution (0.25 U/µl, not provided) to the membrane center of the RNA Binding Column. Incubate the column on the benchtop for 15 mins.
-Note: After incubation, do not perform centrifuge; please proceed step 4-1-d directly.
- 4-1-d. Add 250 µl of Wash Buffer R1 to the RNA Binding Column. Centrifuge at 18,000 xg for 30 secs. Discard the flow-through and return the RNA Binding Column back to the Collection Tube.
- 4-1-e. **After DNase I treatment, proceed the step 4-3.**

- 4-2. Add 500 µl of Wash Buffer R1 to the RNA Binding Column. Centrifuge at 18,000 xg for 30 secs. Discard the flow-through and return the RNA Binding Column back to the Collection Tube.
-Note: Make sure that ethanol has been added into Wash Buffer R1 at the first use.
- 4-3. Add 500 µl of Wash Buffer R2 to the RNA Binding Column. Centrifuge at 18,000 xg for 30 secs. Discard the flow-through and return the RNA Binding Column back to the Collection Tube.
-Note: Make sure that ethanol has been added into Wash Buffer R2 at the first use.
- 4-4. Repeat step 4-3 for one more washing.

5. Dry column

- 5-1. Centrifuge the RNA Binding Column at 18,000 xg for 3 mins to dry the RNA Binding Column.
-Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.

6. Elution

- 6-1. Place the RNA Binding Column to an Elution Tube (provided, 1.5 ml microcentrifuge tube).
- 6-2. Add 30~50 µl of RNase-free ddH₂O to the membrane center of the RNA Binding Column. Stand the RNA Binding 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 (<30 µl). It will lower the RNA yield.
- 6-3. Centrifuge the RNA Binding Column at 18,000 xg for 1 min to elute RNA. Store the extracted RNA at -70°C.

Problem shooting:

Problem/Possible Reason/Solution

Little or no RNA eluted

Poor sample lysis because of insufficient mixing with Lysis Buffer CX
Mix the sample and Lysis Buffer CX immediately and thoroughly by pulse-vortexing 10 secs.

Poor sample lysis because of insufficient Proteinase K activity
1. Make sure the reactive temperature and time is correct.
2. Do not add Proteinase K into Lysis Buffer CX directly.

Insufficient lysis time
Make sure the sample has been incubated at R.T. for 30 mins after mixing with Lysis Buffer CX and Proteinase K.

Poor sample lysis because of too much sample be used
Reduce the sample size or increase the volume of Lysis Buffer CX and 70% ethanol proportionally.

Using bad quality blood
1. Fresh blood is always recommended.
2. Make sure blood is collected in a standard blood collection tube (e.g., EDTA tube) and be stored at -70°C.

Kit stored under improper conditions
All components of FavorPrep™ Whole Blood Total RNA Mini Kit should be stored 15~25°C.

RNA is not completely eluted
Add RNase-free ddH₂O onto the membrane center of the RNA Binding Column, stand the column until RNase-free ddH₂O has been absorbed completely.

Improper preparation of the Wash Buffer R1 and Wash Buffer R2
Make sure that correct amount of ethanol has been added to Wash Buffer R1 and Wash Buffer R2 at the first use.

RNA is degraded

Exceed cells in the sample
Reduce the sample size.

RNase contamination
Make sure the environment is RNase-free. Use disposable RNase-free plasticware.

Sample stored under improper conditions
Flash freeze fresh samples (cultured cells) in liquid nitrogen and store at -80°C, if the sample is not been treated immediately.

Ethanol contains RNases
Make sure that the ethanol be used is RNase free grade.

Sample is old or not stored well
Make sure that sample blood is fresh and stored well.

DNA contamination

The activity of DNase I is insufficient
Use a fresh or well-stored DNase I and reaction buffer.

A260/A280 ration of eluted total RNA is low

Use acidic pH of ddH₂O to elute or dilute RNA
Use acidic 10 mM of Tris-HCl or TE buffer to elute or dilute RNA samples.

Poor performance in downstream applications

Eluted RNA with ethanol residue
Make sure the Dry Column Step "centrifugation for 3 mins" has been done after washing the RNA Binding Column.