User Manual

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).

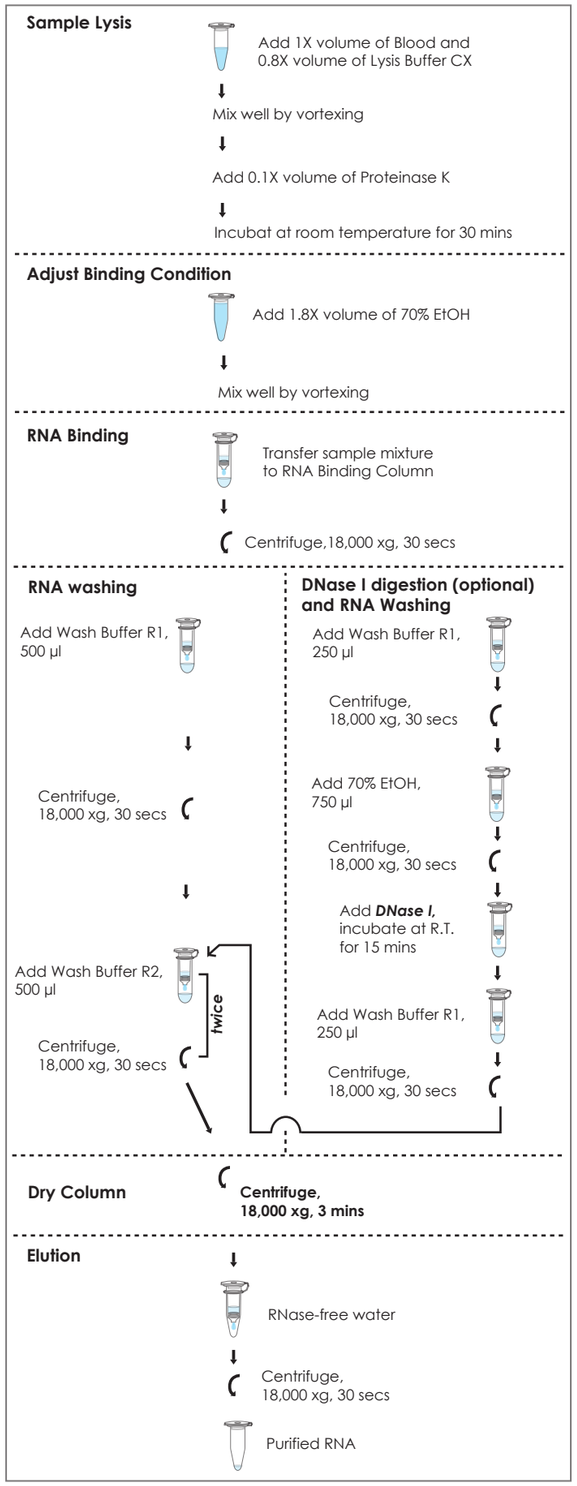
Quality Control:

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: up to 100 µg/RNA Binding Column
8. Column applicability: centrifugation and vacuum
9. Minimum elution volume: 20 µl/RNA Binding Column

Procedure Overview:



Product description:

FavorPrep™ Whole Blood Total RNA Mini Kit is designed for isolation of total RNA from whole blood RNA and prevention of RNA degradation during the isolation procedure. The technology using a chaotropic salt buffer to lyses the cells, inactive the RNase and binds RNA (>200 nt, e.g., 18S, 28S RNA, pri-miRNA) to the silica membranes of the RNA Binding Column. With the on-column DNase I digestion for further DNA removal and membrane washed by 2 wash buffers. The highly pure RNA is eluted from the membrane in a low-ionic-strength buffer and are captured in a Elution Tube. This extracted total RNA can be used directly for the downstream applications such as Real-time RT-PCR, cDNA synthesis, Northern blotting, primer extension and mRNA selection etc.

Additional materials required

- Pipets and pipet tips, sterile (nuclease-free)
- RNase-free 96~100% ethanol (for preparation of Wash Buffer)
- RNase-free 70% ethanol
- Crushed ice
- RNase-free DNase I and DNase I reaction buffer

Preparation of working buffers:

1. **Working Wash Buffer**
   Add RNase-free ethanol to Wash Buffer R1 and Wash Buffer R2 at the first use. Store the buffers at room temperature (15~25°C).
2. **Working DNase I reaction solution (*for Optional Step*)**
   Dilute RNase-free DNase I in DNase I reaction buffer (1 M NaCl, 10 mM $MnCl_2$, 20 mM Tris-HCl, pH 7.0 at 25°C) to final conc. 0.25 U/µl. Stored at 4°C before use.

Important note:

1. Make sure the workstation is RNase-free when handling RNA.
2. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
3. Add ethanol (RNase-free, 96~100%) to Wash Buffer R1 and Wash Buffer R2 at the first use.
4. Prepare working DNase I solution (for optional step: Digest DNA by DNase I) before starting the isolation procedure.
5. The eluted RNA should immediately be kept on ice. For long-term storage, freeze it at -70°C.

Safety Information:

- ***CAUTION:*** Lysis Buffers CX and Wash Buffer R1 contain guanidinium salts which can form highly reactive compounds when combined with bleach. ***DO NOT add bleach or acidic solutions directly to the waste liquid.***

Kit Component: **Lysis Buffer CX**

Hazard contents
Guanidinium thiocyanate
CAS-No. 593-84-0
EC-No. 209-812-1

Hazard statement(s)

H302 + H312 + H332	Harmful if swallowed, in contact with skin or if inhaled.
H314	Causes severe skin burns and eye damage.
H412	Harmful to aquatic life with long lasting effects.

Precautionary statement(s)

P260	Do not breathe dust/ fume/ gas/ mist/ vapours/ spray.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301 + P312 + P330	IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth.
P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
P304 + P340 + P310	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Kit Component: **Wash Buffer R1**

Hazard contents
Guanidine hydrochloride
CAS-No. 50-01-1
EC-No. 200-002-3

Hazard statement(s)

H302 + H332	Harmful if swallowed or if inhaled.
H315	Causes skin irritation.
H319	Causes serious eye irritation.

Precautionary statement(s)

P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P301 + P312 + P330	IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

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 20~50 µl of RNase-free ddH_2O 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 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 (<20 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 extacted 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 ddH_2O onto the membrane center of the RNA Binding Column, stand the column until RNase-free ddH_2O 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 ddH_2O 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 "centrifigation for 3 mins" has been done after washing the RNA Binding Column.