

Kit Contents:

Cat. No.	FAFRK000B_Micro (4 preps)	FAFRK001B_Micro (100 preps)
Bead tube with glass bead	4 pcs	100 pcs
Lysis Buffer S1	3.5 ml	80 ml
Binding Buffer S2	1.8 ml	45 ml
IR Buffer S3	0.8 ml	15 ml
Wash Buffer 1 (concentrate)*	1.3 ml	44 ml
Wash Buffer 2 (concentrate)**	1.5 ml	35 ml
RNase-free ddH ₂ O	1 ml	15 ml
Binding Column B1	4 pcs	100 pcs
Binding Column R2	4 pcs	100 pcs
MicroElute Column Z3 (blister packaging)	4 pcs	10 pcs × 10
Collection Tube	12 pcs	100 pcs × 3
Elution Tube	4 pcs	100 pcs
User manual	1	1

※ Store the MicroElute Column Z3 to 4 ~ 8°C upon receipt

Cat. No.	FAFRK000B_Micro	FAFRK001B_Micro
* Ethanol volume for Wash Buffer 1	0.5 ml	16 ml
** Ethanol volume for Wash Buffer 2	6 ml	140 ml

Specification:

Principle: bead beating and spin column (silica membrane)

Sample: up to 250 mg

Yield: up to 5 µg

Minimum elution volume: 10 µl

Required material to be provided by user

Pipettors and RNase- free pipet tip

Microcentrifuge and RNase-free 1.5 ml microcentrifuge tube

Vortex

Ethanol (96~100%)

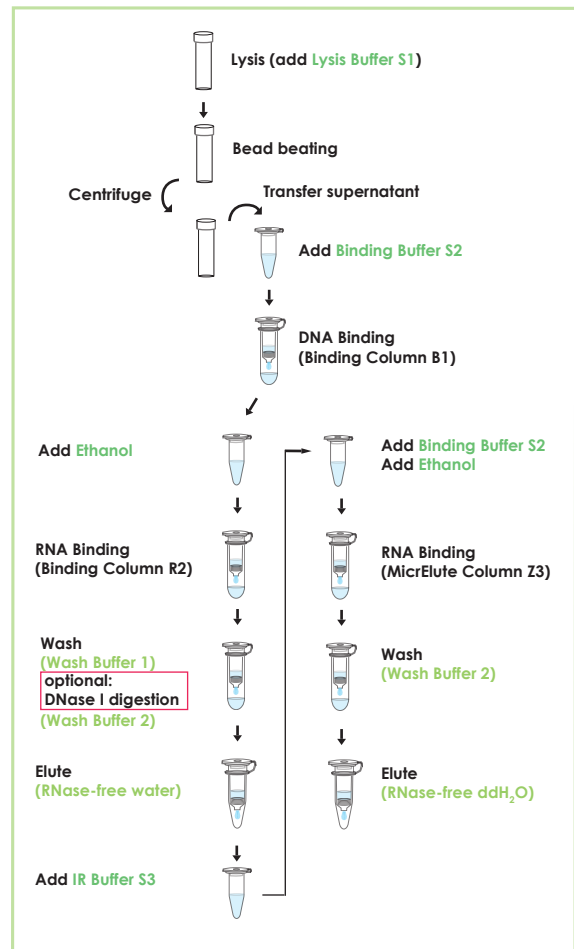
14.3 M β-mercaptoethanol (β-ME)

RNase-free DNase I and DNase I reaction buffer (1M NaCl, 10 mM MnCl₂, 20 mM Tris-HCl, pH 7.0)

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. Pipet a required volume of Lysis Buffer S1 to another RNase-free container and add 10 µl β-mercaptoethanol (β-ME) per 1 ml Lysis Buffer S1 before use.
5. Add required volume of RNase-free ethanol (96~100%) to Wash Buffer 1 and Wash Buffer 2 when first use.
6. Dilute RNase-free DNase I in reaction buffer (1 M NaCl, 10 mM MnCl₂, 20 mM Tris-HCl, pH 7.0 at 25°C) to final conc. 0.2 U/µl.

Brief Procedure:



General Protocol:

Please Read Important Notes Before Starting The Following Steps.

1. Transfer up to 250 mg of soil or stool sample into Bead tube.
2. Add 600 μ l of **Lysis Buffer S1** (β -mercaptoethanol added) to the sample, Secure the cap and vortex the tube at maximum speed for 5 minutes.
3. Centrifuge at full speed (\sim 18,000 x g) for 1 minute.
4. Transfer the clarified supernatant up to 350 μ l to a clean 1.5 ml centrifuge tube (not provided) and measure the volume of the clarified supernatant.
-- Note! Do not pipet any float or debris when transfer the clarified supernatant.
5. Add 1 volume of **Binding Buffer S2** to the clarified supernatant and mix well by vortexing for 10 seconds. Place a **Binding Column B1** in a Collection tube and transfer the sample mixture to the **Binding Column B1**.
6. Centrifuge the Binding Column B1 at 4,000 x g for 30 seconds. **After the centrifugation, do not discard the flow-through.** Transfer the flow-through inside the Collection Tube to a clean 1.5 ml centrifuge tube (not provided) and measure the volume of the flow-through. Discard the Binding Column B1.
7. Add 1 volume of **96 ~ 100% ethanol** and mix well by vortexing for 10 seconds. Place a **Binding Column R2** in a Collection tube and transfer the sample mixture (up to 750 μ l) to the **Binding Column R2**. Centrifuge the Binding Column R2 at 4,000 x g for 30 seconds. Discard the flow-through and place the Binding Column R2 back to Collection Tube.
8. Repeat Step 7 for the rest of the sample mixture.
9. (Optional): To eliminate genomic DNA contamination, follow the steps from 9a. Otherwise, proceed to Step 10 directly.
 - 9a. Add 200 μ l of **Wash Buffer 1** to the Binding Column R2. Centrifuge at full speed (\sim 18,000 x g) for 30 seconds then discard the flow-through. Place the Binding Column R2 back to the Collection Tube.
-- Make sure that ethanol (96~100%) has been added into Wash Buffer 1 when first use.
 - 9b. Add 30 μ l of **RNase-free DNase I solution** (0.2 U/ μ l, not provided) to the membrane center of Binding Column R2. Place the Column on the benchtop for 15 minutes.
 - 9c. Add 200 μ l of **Wash Buffer 1** to the Binding Column R2. Centrifuge at full speed (\sim 18,000 x g) for 30 seconds then discard the flow-through. Place the Binding Column R2 back to the Collection Tube.
 - 9d. After DNase I treatment, proceed to step 11.
10. Add 400 μ l of **Wash Buffer 1** (ethanol added) to the Binding Column R2. Centrifuge at full speed (\sim 18,000 x g) for 30 seconds. Discard the flow-through and place the Binding Column R2 back to the Collection Tube.
-- Make sure that ethanol (96~100%) has been added into Wash Buffer 1 when first use.
11. Add 700 μ l of **Wash Buffer 2** (ethanol added) to the Binding Column R2. Centrifuge at full speed (\sim 18,000 x g) for 30 seconds. Discard the flow-through and place the Binding Column R2 back to the Collection Tube.
-- Make sure that ethanol (96~100%) has been added into Wash Buffer 2 when first use.
12. Centrifuge the Binding Column R2 at full speed (\sim 18,000 x g) for an additional 2 minutes to dry the column.
13. Place the Binding Column R2 to a clean 1.5 ml centrifuge tube (not provided) and add 100 μ l of **RNase-free ddH₂O** to the membrane center of the Binding Column R2. Stand the Binding Column R2 for 30 seconds.
-- Make sure that the RNase-free ddH₂O is dispensed onto the membrane center and is absorbed completely.
14. Centrifuge the Binding Column R2 at full speed (\sim 18,000 x g) for 30 seconds to elute RNA. Add 100 μ l of **IR Buffer S3** to the eluate sample, mix well by vortexing for 10 seconds. Incubate the sample mixture at room temperature for 2 minutes.
-- Note: IR Buffer S3 must be suspended completely by vigorously vortexing before every using.
-- Cut off the end of pipet tip to make it easier for pipetting the IR Buffer S3.
15. Centrifuge the mixture at full speed (\sim 18,000 x g) for 1 minutes. Carefully transfer the clarified supernatant to a 1.5 ml microcentrifuge tube (not provided) and measure the volume of the clarified supernatant.
--Avoid pipetting any debris and pellet.
16. Add 1 volume of **Binding Buffer S2** and 1 volume of 96 ~ 100% ethanol to the clarified supernatant from step 15. Mix well by vortexing. Place a **Binding Column Z3** in a Collection tube. Transfer the sample mixture to the Binding Column Z3. Centrifuge the Binding Column Z3 at 4,000 x g for 30 seconds. Discard the flow-through and place the Binding Column Z3 back to Collection Tube.
17. Add 700 μ l of **Wash Buffer 2** (ethanol added) to the Binding Column Z3. Centrifuge at full speed (\sim 18,000 x g) for 30 seconds. Discard the flow-through and place the Binding Column Z3 back to Collection Tube.
18. Centrifuge at full speed (\sim 18,000 x g) for an additional 2 min to dry the column.
-- Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.
19. Place the **Binding Column Z3** to an **Elution Tube** (provided) and add 10 μ l of **RNase-free ddH₂O** to the membrane center of Binding Column Z3
--make sure that the RNase-free ddH₂O is dispensed onto the membrane center and is absorbed completely.
20. Centrifuge at full speed (\sim 18,000 X g) for 1 minute to elute RNA. Store RNA at -70°C.