FavorPrep™ Soil/Stool RNA Extraction MicroElute Kit

For Research Use Only

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 ddH2O	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

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* 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

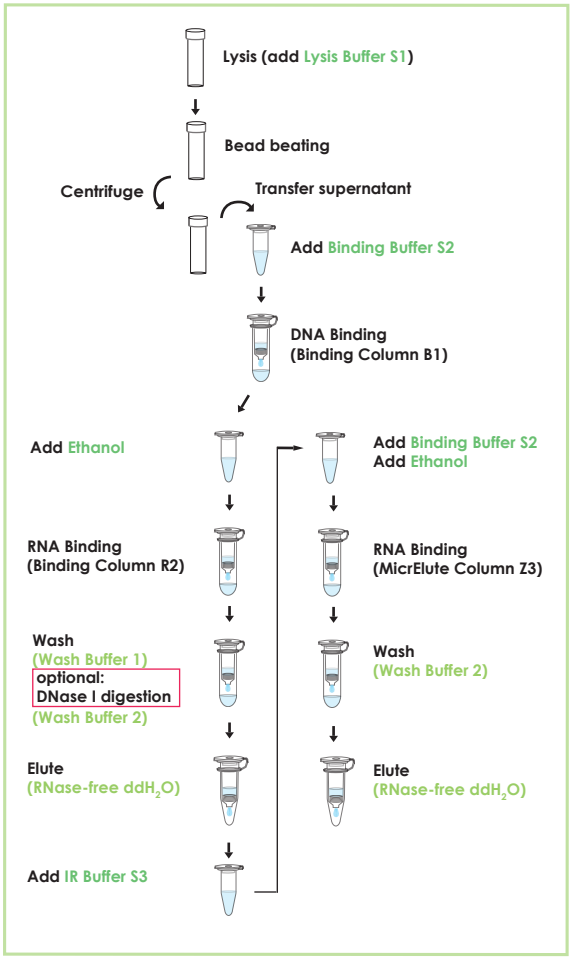
Required material to be provided by user

1. Pipettors and RNase- free pipet tip
2. Microcentrifuge and RNase-free 1.5 ml microcentrifuge tube
3. Vortex
4. RNase-free 96~100% Ethanol
5. RNase-free 70% Ethanol (optional)
6. 14.3 M ß-mercaptoethanol (ß-ME)
7. RNase-free DNase I reaction buffer (1M NaCl, 10 mM $MnCl_2$, 20 mM Tris-HCl, pH 7.0) and make the final concentration of DNase I to 0.25 U/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 hese buffers.
4. Pipet a required volume of Lysis Buffer S1 to another RNase-free container and add 10 µl ß-mercaptoethanol (ß-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. 0.25 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 µl of **Lysis Buffer S1** (ß-mercaptoethanol added) to the sample, Secure the cap and vortex the tube at maximum speed for 5 minutes.
3. Centrifuge at full speed (~18,000 xg) for 1 minute.
4. Transfer the clarified supernatant up to 350 µl 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 xg for 30 seconds. ***After the centrifugation, do not discard the flow-though.*** 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 µl) to the **Binding Column R2**. Centrifuge the Binding Column R2 at 4,000 xg for 30 seconds. Discard the flow-through and place the Biding 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 µl of **Wash Buffer 1** to the Binding Column R2. Centrifuge at full speed (~18,000 xg) 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 750 µl of **RNase-free 70% Ethanol** to the Binding Column R2. Centrifuge at full speed (~18,000 xg) for 30 seconds then discard the flow-through. Place the Binding Column R2 back to the Collection Tube.
   9c. Add 60 µl of **RNase-free DNase I solution** (0.25 U/µl, not provided) to the membrane center of Binding Column R2. Place the Column on the benchtop for 15 minutes.
   9d. Add 200 µl of **Wash Buffer 1** to the Binding Column R2. Centrifuge at full speed (~18,000 xg) for 30 seconds then discard the flow-through. Place the Binding Column R2 back to the Collection Tube.
   9e. After DNase I treatment, proceed to step 11.
10. Add 400 µl of **Wash Buffer 1** (ethanol added) to the Binding Column R2. Centrifuge at full speed (~18,000 xg) for 30 seconds. Discard the flow-through and place the Biding 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 µl of **Wash Buffer 2** (ethanol added) to the Binding Column R2. Centrifuge at full speed (~18,000 xg) for 30 second. 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 (~18,000 xg) for an additial 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 µl of **RNase-free ddH_2O** to the membrane center of the Binding Column R2. Stand the Binding Column R2 for 30 seconds.
    -Make sure that the RNase-free ddH_2O is dispensed onto the membrane center and is absorbed completely.
14. Centrifuge the Binding Column R2 at full speed (~18,000 xg) for 30 seconds to elute RNA. Add 100 µl 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 vrotexing 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 (~18,000 xg) for 1 minute. 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 xg for 30 seconds. Discard the flow-through and place the Biding Column Z3 back to Collection Tube.
17. Add 700 µl of **Wash Buffer 2** (ethanol added) to the Binding Column Z3. Centrifuge at full speed (~18,000 xg) for 30 seconds. Discard the flow-through and place the Biding Column Z3 back to Collection Tube.
18. Centrifuge at full speed (~18,000 xg) for an additional 2 minutes 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 µl of **RNase-free ddH_2O** to the membrane center of Binding Column Z3
    -Make sure that the RNase-free ddH_2O is dispensed onto the membrane center and is absorbed completely.
20. Centrifuge at full speed (~18,000 xg) for 1 minute to elute RNA. Store RNA at -70°C.