SPINeasy™ DNA/RNA Kit for Feces

Cat. No.: 116555050 (50 PREPS)/116555000 (5 PREPS)

Quick-Start Protocol

Revision Aug 2023

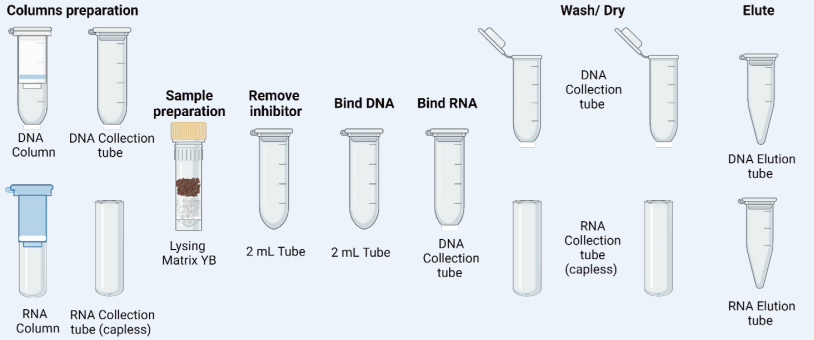
This protocol is designed to extract microbial DNA and RNA from the same feces sample.

Scan QR code for more information from instruction manual

Notes before starting:

- Phenol:Chloroform:Isoamyl Alcohol (25:24:1, pH 6.5-8.0) is required for this protocol.
- The protocol requires Ethanol (96–100%) that needs to be supplied by user. Do not use denatured alcohol.
- Buffer FDR2 needs to be stored at 2-8˚C upon reception.
- For faster processing, it is advised to pre-position the plasticwares and columns as shown below.

Plasticware and Column required for SPINeasy™ DNA/RNA Kit for Feces



Column and sample preparation

1. Transfer the **DNA Column** (capless) into a **DNA Collection tube** (provided). Add **200 µL Equilibration Buffer** to both **DNA** (capless) and **RNA (blue) Column** membranes to ensure their performances.

   Wait at least **1 min** and centrifuge for **30 sec @ maximum speed**. Transfer the Columns into new Collection tubes (provided). The treated Columns can be stored at 2-8˚C for a few days if required.

2. Weight up to **250 mg** of feces and add it into **Lysing Matrix YB** tube.

3. Add **100 µL** Phenol:Chloroform:Isoamyl Alcohol and **650 µL Buffer FDR1** to the Lysing Matrix YB tube.

   Homogenize using vortex at **2,500-2,700 rpm** for **20 min** or **Fastprep® 35 sec, 5m/sec.** Centrifuge for **2 min @ maximum speed.**

Remove inhibitor

4. Transfer the supernatant (~400-600 µL) into a **2 mL Tube** (provided).

   Optional: *The Lysing Matrix YB tube may be re-centrifuged after transfer of the supernatant, to recover any additional lysate using a tip.*

   Add **200 µL Buffer FDR2** to precipitate contaminants, vortex for **1 sec** and centrifuge for **2 min @ maximum speed.**

Bind DNA / RNA

5. **DNA and RNA binding**

   Transfer the supernatant into a 2 mL Tube (provided). Add 1 volume **Buffer FDR3 (1:1)** and vortex for **1 sec**. For example, for 700 µL supernatant, add 700 µL Buffer FDR3.

   - The treated DNA Column (capless) shall be loaded in 2 steps as follow:

a) Pour ~**750 µL** of the lysate into the DNA Column (capless). Centrifuge for **10 sec @ 15,000 *g***. The 2 mL Tube including the remaining lysate mixed with Buffer FDR3 can be centrifuged together to recover any mixture found on the tubing lid and wall.

   - Transfer the DNA Column (capless) into a new DNA Collection tube (provided) and close the lid of the tube containing the first flow-through (it will be used for RNA isolation).

b) Load the remaining lysate to the DNA Column (capless) and centrifuge for **10 sec @ 15,000 *g***.

   - Transfer the DNA Column (capless) into a new DNA Collection tube (provided) and combine the second flow-through to the first flow-through for RNA purification.

RNA purification

Add **250 µL Ethanol** (not provided) into the lysate flow-through and mix immediately by pipetting up and down or vortex for **1 sec.**

Pour ~**825 µL** of the obtained supernatant into the RNA Column (**blue**), centrifuge for **10 sec @ 15,000 *g*** and discard the flow-through.

Optional: *The DNA Collection tube including the lysate-FDR3-Ethanol mix may be centrifuged together to recover any mixture found on the tubing lid and wall.*

Repeat until all the lysate has been processed through the RNA Column (**blue**). Transfer the RNA Column (**blue**) to a new **RNA Collection tube** (provided).

DNA purification

If RNA and DNA is required, close the lid of the DNA Column (capless) to prevent it from drying out (until step 5 is performed). The DNA Column (capless) can also be stored at 2-8˚C for extended storage.

Wash

6. **1st wash.** Add **600 µL Buffer FDR4/DNA** to the center of the DNA Column and **600 µL Buffer FDR4/RNA** to the center of the RNA Column. Centrifuge for **10 sec @ 15,000 *g***, discard the flow-through and place the column back into the same Collection tube.
7. **2nd wash.** Add **600 µL Buffer FDR5** to the center of the column and centrifuge for **30 sec @ 15,000 *g***, discard the flow-through and place the column back into the same Collection tube.
8. **Dry.** Transfer the column into new DNA and/or RNA Collection tube (provided) and centrifuge for **1 min @ maximum speed.**

Elute

9. Transfer the column into new **DNA** and/or **RNA Elution tube** (provided). Add **100 µL RNase Free Water** directly into the column membrane, wait for **1 min** and centrifuge for **1 min @ maximum speed.** The RNA/DNA samples are now ready for downstream applications.

   Note: *Eluting with 100 µL RNase Free Water will maximize nucleic acid yield. For more concentrated sample, a minimum of 50 µL RNase Free Water can be used.*

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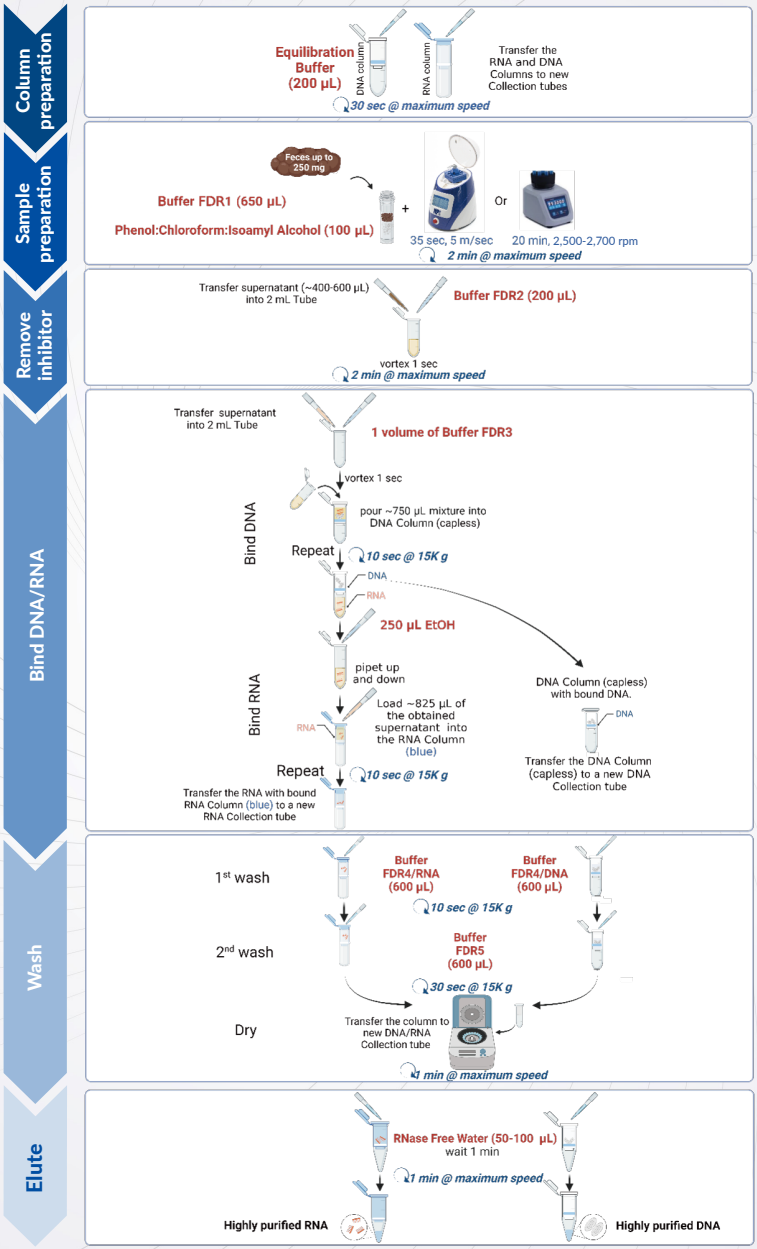
Flow-Chart



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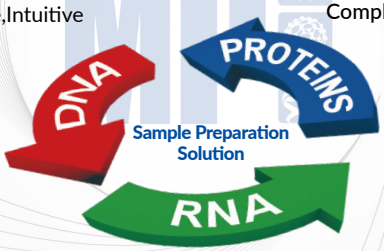

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Learn more at www.mpbio.com