Protocol of RNase-Free DNase Set (Vazyme #RH104-C1)

Product Description

RNase-Free DNase Set is for DNase treatment when using with Vazyme RNA purification kits.

Components

Components	RH104-C1 (50 rxns)
DNase I	550 μl (1550 U)
Buffer RDD	4 ml

Storage

Store DNase I at -30 ~ -15°C and Buffer RDD at room temperature. Ship at ≤ 0 °C.

Experiment Process

1. Preparation of DNase I working solution: For each reaction, take 10 μl DNase I, add 70 μl Buffer RDD, and gently mix.

▲ Working solution is recommended to be prepared freshly.

2. Follow the operation steps of the RNA extraction kit as below, add 350 μl Wash Buffer to the FastPure RNA Columns that has already bound RNA. Centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the filtrate.

3. Add 80 μl DNase I working solution prepared in step 1 to the center of the spin column, and let it stand at room temperature (20-30 °C) for 15 - 25 min.

4. Add 350 μl Wash Buffer to the spin column, centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the filtrate. Continue following the RNA extraction kit operation steps.

Taking RH104-C1 in combination with Vazyme #RC112 as an example:

1. Cells: Add 500 μl Buffer RL to every <5 × 10^6 cells and vortex until there are no visible clumps of cells.

2. Tissues: Add 500 μl (350 μl for liver tissue) Buffer RL to every 10 - 20 mg of fresh tissue. Disrupt the tissue and homogenize the lysate until there are no visible tissue pieces.

3. Transfer the lysate to FastPure gDNA-Filter Columns III (FastPure gDNA-Filter Columns III had been put into the collection tube) and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the FastPure gDNA-Filter Columns III and collect the filtrate.

4. Add absolute ethanol (0.5 × the volume of filtrate) to the filtrate and mix thoroughly. For liver tissue, add 50% ethanol (1 × the volume of filtrate).

5. Transfer the mixture from Step 3 to FastPure RNA Columns III (FastPure RNA Columns III

had been put into the collection tube) and centrifuge at 12,000 rpm (13,400 × g) for 30 sec and discard the filtrate.

6. Add 350 µl Buffer RW1 to the FastPure RNA Columns III and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the filtrate.

7. Add 10 µl DNase I to 70 µl Buffer RDD. Mix by gently inverting the tube. Centrifuge briefly.

8. Add 80 µl DNase I working solution prepared in FastPure RNA Columns III, and incubate at room temperature (20 - 30 °C) for 15 - 25 min.

9. Add 350 µl Buffer RW1 to the FastPure RNA Columns III and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the filtrate.

10. Add 700 µl Buffer RW2 (check if absolute ethanol has been added in advance!) to the FastPure RNA Columns III and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the filtrate.

11. Add 500 µl Buffer RW2 (check if absolute ethanol has been added in advance!) to the FastPure RNA Columns III and centrifuge at 12,000 rpm (13,400 × g) for 2 min. Carefully remove the adsorption column from the collection tube to avoid contacting with the filtrate to prevent contamination.

12. (Optional) If the adsorption column has residual liquid or contacts with the filtrate, discard the filtrate and place the FastPure RNA Columns III back into the collection tube. Centrifuge at 12,000 rpm (13,400 × g) for 1 min to prevent ethanol contamination.

13. Transfer the adsorption columns to new RNase-free Collection Tubes 1.5 ml carefully. Add 50 - 200 µl of RNase-free ddH_2O to the center of the adsorption column without touching the membrane and incubate at room temperature for 1 min. Centrifuge at 12,000 rpm (13,400 × g) for 1 min to elute the RNA.

Taking RH104-C1 in combination with Vazyme #RC411 as an example:

1. Determine sample input amount and appropriate lysis buffer according to 08-2/Lysis Buffer Selection in the Manual. Take the correct amount of plant tissues ground by liquid nitrogen and immediately add 600 µl Buffer EL or 600 µl Buffer PSL. Mix the samples and lysis buffer thoroughly by vortexing vigorously for 30 sec. Centrifuge at 12,000 rpm (13,400 × g) for 5 min, then perform subsequent operations immediately.

▲ After the samples are taken out from liquid nitrogen, immediately add it to the lysis buffer and mix well. Do not place samples at room temperature. Sample thawing or uneven mixing of the sample and lysis buffer will lead to RNA degradation.

▲ Plant tissues with high starch contents (such as potatoes, sweet potatoes, seeds, etc.) react with lysis buffer at room temperature to produce gelatinous substances, and the longer the contact time, the more gelatinous substances produced. Therefore, samples should be centrifuged as soon as possible after lysis, and transfer the supernatant to FastPure gDNA-Filter Columns III. When taking the supernatant, do not absorb the gelatinous substances to avoid clogging FastPure gDNA-Filter Columns III.

▲ A small amount of floating matter in the supernatant after centrifugation is normal and the subsequent

experiments can be carried out directly

2. Transfer about 500 μl supernatant to FastPure gDNA-Filter Columns III (FastPure gDNA-Filter Columns III has been put into the collection tube) and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the FastPure gDNA-Filter Columns III and collect the filtrate.

▲ The pipetted volume of supernatant can be adjusted according to the actual situation.

▲ FastPure gDNA-Filter Columns III has a good filtering effect on impurities, so absorbing a small number of sample fragments will not affect subsequent experiments.

3. Add 0.5 times the filtrate volume of absolute ethanol (about 250 μl and it can be adjusted according to the actual situation) to the collection tube and mix well by vortexing for 15 sec.

▲ It is normal to appear turbidity or flocculent precipitate after adding ethanol, and the mixture (including flocculent matter) can be continued for subsequent operations.

4. Transfer the mixture from step 3 to FastPure RNA Columns V (FastPure RNA Columns V has been put into the collection tube) and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. **Discard the filtrate**.

▲ The adsorption column volume is 750 μl. If the volume of the mixture exceeds 750 μl, please transfer the solution in several times.

5. Add 700 μl Buffer RWA to the FastPure RNA Columns V and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the filtrate.

6. **Preparation of DNase I working solution:** For each reaction, take 10 μl DNase I, add 70 μl Buffer RDD, and gently mix.

▲ Working solution is recommended to be prepared freshly before use.

7. Add 80 μl DNase I working solution to the center of the spin column, and incubate at room temperature (20 ~ 30 °C) for 15 - 25 min.

8. Add 350 μl Buffer RWA to the spin column, and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the filtrate.

9. Add 500 μl Buffer RWB (check if absolute ethanol has been added in advance!) to the FastPure RNA Columns V and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the filtrate.

10. Repeat step 9.

11. Place the FastPure RNA Columns V back into the collection tube and centrifuge at 12,000 rpm (13,400 × g) for 2 min.

12. Transfer the FastPure RNA Columns V to new RNase-free Collection Tubes 1.5 ml. Add 30 - 100 μl RNase-free ddH_2O to the center of the adsorption column without touching the membrane and centrifuge at 12,000 rpm (13,400 × g) for 1 min.