HiPure Yeast & MicroBiol RNA Kit

Introduction

This kit uses phenol and bead grinding method, which is suitable for extracting high purity total RNA from yeast, bacteria, fungal mycelium or sporangium powder, and other difficult lysed microorganisms. The kit is based on silica gel column purification technology, the whole extraction process only takes about 40 minutes. The purified RNA can be directly used for RT-PCR, Northern blot, Poly-A + purification, nucleic acid protection and in vitro translation experiments, ect.

Kit Contents

Product Number	R418201	R418202	R418203
Purification Times	10 preps	50 preps	250 preps
gDNA Filter Mini Column	10	50	250
HiPure RNA Mini Column I	10	50	250
2ml Collection Tubes	20	100	500
2ml Bead Tubes F	10	50	250
Buffer ATL	8 ml	30 ml	150 ml
Buffer PCI	8 ml	30 ml	150 ml
Buffer GDP	5 ml	20 ml	100 ml
Buffer RW2*	6 ml	20 ml	2 x 50 ml
RNase Free Water	1.8 ml	10 ml	30 ml

Storage and Stability

Buffer PCI should be stored at 2–8°C upon arrival. However, short-term storage (up to 12 weeks) at room temperature (15–25°C) does not affect their performance. The remaining kit components can be stored at room temperature (15–25°C) and are stable for at least 18 months under these conditions.

Materials and Equipment to be Supplied by User

- absolute ethyl alcohol(96-100%)
- Dilute Buffer RW2 with 24ml (10 Preps), 80ml (50 Preps) or 2 x 200ml (250 Preps) absolute ethanol and store at room temperature.

Protocol : Total RNA Extraction from Yeast and Microbe

1. Centrifuge to collect microbial cells.

- Liquid culture: transfer 0.5~1.8ml in exponential growth phase bacteria, yeast or fungus culture to the 2ml Bead Tubes F. Centrifuge at 12,000 x g for 3 minutes to collect the cells. Remove the liquid culture

  Bacteria: use cells $\leqslant$ 1 x 10^9 or wet weight $\leqslant$30mg.

  Yeast or fungus: use cells $\leqslant$ 1 x 10^7 or wet weight $\leqslant$100mg.

- Solid medium: scrape and wash the mycelium from the solid medium with 1.5~1.8ml normal saline or PBS. Then transfer to the 2ml Bead Tubes F. Centrifuge at 12,000 x g for 3 minutes to collect the cells. Remove the supernatant.
- Sporangium powder or fungus powder: transfer 30~100mg sporangium powder or fungus powder to the 2ml Bead Tubes F.
- Bulk volume fluid (low cells concentration): transfer 1~1.8ml serum, plasma, body effusion, culture supernatant, liquidfy sputum fluid, secretion fluid, urine, lavage fluid, saliva, ect to the 2ml Bead Tubes F. Centrifuge at 12,000 x g for 10 minutes to collect the cells. Remove the supernatant.

2. Add 450µl Buffer ATL and 450µl Buffer PCI to the sample and screw the lid tightly. Vortex at maximum speed for 10 minutes or place on a bead grinding machine for fast grinding with 30~60 seconds.

- PowerLyzer grinder: recommend 2000rpm for 30s, pause for 30s and then repeat once.
- FastPrep 24 grinder: recommend 5m/s for 30s, pause for 30s, and then repeat once.
- Tissue Lysis II grinder: recommend 25Hz for 5min, reposition and then repeat once.

3. Centrifuge the bead tubes at 12,000 x g for 5 minutes at room temperature.

4. Transfer 300µl supernatant to a new centrifuge tube, add 300µl Buffer GDP to the tube, invert

up side down for 6~8 times to mix.

5. Place the gDNA Filter Mini Column in a 2ml collection tube and transfer all mixture to the Column. Centrifuge at 12,000 x g for 30~60 seconds.

6. Discard the gDNA Filtre Mini Column, add 180µl isopropanol to the filtrate, pipet 3~5 times to mix.

7. Place the HiPure RNA Mini Column to a new 2ml collection tube and transfer all mixture to the column. Centrifuge at 12,000 x g for 30~60 seconds.

8. Discard the filtrate and install the column back into the collection tube. Add 500µl Buffer RW2 to the column. Centrifuge at 12,000 x g for 30~60 seconds.

9. Repeat Step 8 once.

10. Discard the filtrate and install the column back into the collection tube, centrifuge at 12,000 x g for 2 minutes.

11. Transfer the column into a new 1.5ml centrifuge tube, add 30~100µl RNase Free Water to the center of the column membrane. Stay at room temperature for 2 minutes. Centrifuge at 12,000 x g for 1 minute.

    The mini elution volume of the column is 30µl, if RNA yield is over 30µg, we recommend to repeat the elution step once.

12. Discard the columns and store RNA at -80 ° C.

RNA integrity and purity testing

Integrity test: Prepare 1.2% agarose gel with 0.5 x TBE electrophoresis buffers. RNA loading is 0.5-1.5μg. Perform 150V electrophoresis for 15 minutes. On the electrophoresis image, two distinct rRNA bands can be seen, with 28S rRNA showing better brightness than 18S rRNA, indicating that the RNA bands are intact and not degraded.

Purity 1: OD260/280 ratio is an indicator for measuring the degree of protein contamination. The OD260/280 ratio of high purity RNA is 2.0, but OD260, OD280, and OD230 are affected by pH values. This product is dissolved in RNase Free Water (DEPC treated water), with a pH fluctuation of 5.5-7.5 and an OD260/280 ratio of 1.9-2.1.

Purity 2: When the total amount of RNA exceeds 10μg, OD260/230 is generally between 1.3-2.2; When the total amount of RNA is between 5-10μg, A260/230 will be between 0.7-2.0; When the total amount of RNA is less than 3μg, A260/230 will be less than 0.6. This is because Buffers contain guanidine isothiocyanate, and the glass filter membrane has water absorption or background adsorption. Guanidine isothiocyanate has a strong absorbance at 230nm, therefore A260/230 is mainly influenced by this guanidine salt rather than originating from the sample. Researches have shown that low concentrations of guanidine isothiocyanate do not affect applications such as reverse transcription, quantitative RT-PCR, and second-generation sequencing. When 260/230 occurs between 0.2-1.0, it can be ignored and directly used for downstream applications such as reverse transcription. On the premise of not blocking the column, increasing the sample and lysis buffer amount appropriately and increasing the nucleic acid concentration (total nucleic acid content>10μg) can significantly improve OD260/230.