HiPure MicroBiol RNA Kit

Introduction

The HiPure MicroBiol RNA Kit is suitable for extracting high purity total RNA from Bacterial culture medium.HiPure Yeast RNA Kit is suitable for extracting high purity total RNA from Yeast culture medium.The kit combines the one-step MagZol Reagent and silica gel column purification technology to complete the extraction of high purity total RNA in only 40 minutes.The obtained RNA can be directly used for RT-PCR, Northern blot, Poly-A + purification, nucleic acid protection and in vitro translation experiments.

Kit Contents

Product Number	R418201	R418202	R418203
Purification Times	10 preps	50 preps	250 preps
HiPure RNA Mini Columns	10	50	250
2ml Collection Tubes	10	50	250
Glass Beads(0.1~0.6mm)	6 g	30 g	150 g
DNase I	120 ul	600 ul	5 x 600 ul
DNase Buffer	1.8 ml	6 ml	30 ml
Protease Dissolve Buffer	1.8 ml	1.8 ml	15 ml
Buffer ATL	10 ml	50 ml	200 ml
Buffer PHC	10 ml	50 ml	200 ml
Buffer GXP2*	6 ml	20 ml	100 ml
Buffer RW1	10 ml	50 ml	250 ml
Buffer RW2*	5 ml	20 ml	2 x 50 ml
RNase Free Water	1.8 ml	10 ml	30 ml
Protocol	1	1	1

Storage and Stability

Buffer PHC should be stored at 2–8°C upon arrival. DNase I should be stored at -20°C. However, short-term storage (DNase I up to 1 weeks, Buffer PHC 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%)
- chloroform
- Dilute Buffer RW2 with 20ml (10 was diluted with anhydrous ethanol and stored at room temperature
- Lysozyme was dissolved in proper Protease Discharge Buffer with the final concentration of 50mg/ mL and stored at -20 ° C.

Protocol 1.Total RNA Extraction from Yeast and Microbe (R4182)

Note：The Protocol　was suitable for the extraction of high purity total RNA from 1x107 yeast cells and 1x 109 bacteria by thermal phenolic method and physical method.

The amount of yeast cells (2 x107)

Yeast growth can be measured with a spectrophotometer.Due to the difference of different instruments and the influence of various growing conditions, it is difficult to give an accurate and reliable relationship between OD value and the number of yeast cells.For example, the OD600 of 2 x107 yeast cells per milliliter, as measured by Beckman Du-40, was 0.125 after a 4-fold dilution.When measured by DU-7400, OD600 is 0.25.Therefore, we recommend using the plate counting method to compare the OD value measured by the instrument.When measuring OD, dilute or concentrate the sample to ensure that the reading is within the confidence interval of 0.05-0.3.Due to the inconsistency of RNA content in different yeasts, it is recommended to start with 2 x 107 yeasts and adjust the amount of yeast according to the yield and purity obtained.

Bacterial dosage (<1 x 109)

Bacterial dosage (<1 x 109) Bacterial growth can be measured using a

spectrophotometer.Due to the difference of different instruments and the influence of various growing conditions, it is difficult to give an accurate and reliable relationship between OD value and the number of bacteria.For example, when a culture medium containing 1 x 109 bacteria per milliliter was diluted four times, OD600 was 0.125 as measured by Beckman Du-40;When measured by DU-7400, OD600 is 0.25.Therefore, we recommend using the plate counting method to compare the OD value measured by the instrument.When measuring OD, dilute or concentrate the sample to ensure that the reading is within the confidence interval of 0.05-0.3.Different culture conditions and the RNA content of different bacteria vary greatly, and we recommend a bacterial dosage of 2 x 108 for the initial extraction, and then adjust according to the results.

Materials and tools need to be prepared

- Anhydrous ethanol (96-100%)
- Buffer RW2 was diluted with anhydrous ethanol and stored at room temperature
- Buffer GXP2 was diluted with anhydrous ethanol and stored at room temperature
- (optional) 2-hydrophobic ethanol

1. Prepare the homogenate tube: Add 0.4~0.5g mixed glass beads and 0.5ml Buffer PHC to a 2ml centrifuge tube.

2.10,000 x g Centrifuge for 3 minutes to collect 1~1.5 mL yeast or other microorganisms, and completely absorb and discard the culture medium.

3. Add 0.5ml Buffer ATL to the sample, vortex resuspend the bacteria, and transfer it to the prepared homogenate tube.

4. Vortex mixture at top speed for 10 minutes.

Warm bath at 5.65oC for 10 minutes, mixed several times upside down, or placed in a metal bath oscillating for 10 minutes.

6. Add 0.5ml chloroform to the sample, swirl and mix for 15 seconds.Centrifuge for 5 min at 12,000 x g.

7. Transfer the supernatant to a new centrifuge tube and add 1.5 times volume of Buffer GXP2 to the supernatant.Vortex and mix for 15 seconds.

8. Place the HiPure RNA Mini Column in a 2ml collection tube.Transfer half the volume of the mixture to the column.Centrifuge 12,000 x g for 30 SEC.

9. Discard the filtrate and install the column back into the collection tube.Transfer the remaining mixture to the column.Centrifuge 12,000 x g for 30 SEC.

10. Discard the filtrate and install the column back into the collection tube.Add 300 l Buffer RW1 to the column.Centrifuge at 12,000 × g for 60 seconds.

11. Discard the filtrate and install the column back into the collection tube.Prepare DNase I reaction solution according to the table below and mix gently.The DNase I reaction solution was added to the center of the membrane of the RNA binding column.Let stand at room temperature for 20 to 30 minutes.

Dosage of components

DNase Buffer (including 60 l

DNase I (20 units/(including l) 10 (including l

12. Add 500 l Buffer RW1 into the column and leave it at room temperature for 10 minutes.Centrifuge at 12,000 × g for 30 seconds.

13. Discard the filtrate and install the column back into the collection tube.Add 500 l Buffer RW2(diluted with ethanol) to the column.Centrifuge at 12,000 × g for 30 seconds.

14. Discard the filtrate and install the column back into the collection tube.Add 500 l Buffer RW2(diluted with ethanol) to the column.Centrifuge at 12,000 × g for 30 seconds.

15. Discard the filtrate and install the column back into the collecting tube.Centrifuge 12,000 × g empty column for 2 minutes and dry the matrix of the column.

16. Transfer the column into a 1.5 mL centrifuge tube.Add 30-50 l RNase Free Water to the center of the column membrane.Let stand at room temperature for 2 minutes.Centrifuge for 1 min at 12,000 × g.Discard the RNA column and store the RNA sample at -80 ° C.