Hangzhou Simgen Biotechnology Co., Ltd.
5th floor, Building No. 4, No. 8 Xiyuan 1st Road, Hangzhou, P. R. China.
Pc: 310030 Tel: +86-571-87381295 Fax: +86-571-87381295

RNA Purification Kit Instructions

Composition

RNA Purification Kit	5 Preps	50 Preps
Cat. No.	5401005	5401050
Spin Columns	5	50
2 ml Collection Tubes	5	50
Buffer L	4 ml	32 ml
Buffer WA (concentrate)	1.9 ml	19 ml
Buffer WBR (concentrate)	1.5 ml	15 ml
RNase-free Water	1.5 ml	2 ml×3
Instructions	1	1

Storage

1. All the reagents and components can be stored for up to 3 years at room temperature (0~30℃). For longer storage, it is recommended to keep at 2-8℃.
2. The product stored at 2~8℃ should be restored to room temperature before use.

Technical Support

R&D Department, Hangzhou Simgen Biotechnology Co., Ltd. E-mail: technical@simgen.cn , Tel: 400-0099-857.

Introduction

This kit is designed to recover concentrated RNA from 100~200 µl enzymatic reaction systems or RNA solutions containing impurities such as salt and phenol/chloroform, glycogen or polysaccharide residues, protein residues, etc. The column purification technology only binds RNA larger than 200 nt to the Spin Column, and substances such as salt, polysaccharide, and protein are filtered out. RNA can eventually be eluted to a tiny volume of 50 µl and can be immediately used in various molecular biology experiments such as Northern blotting, RT-PCR, and chip analysis.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol, RNase-free water maybe required.
2. 1.5 ml centrifuge tubes (DNase-free & RNase-free 1.5 ml centrifuge tubes must be used).
3. Pipettes and pipette tips (DNase-free & RNase-free pipette tip with filter must be selected).
4. Disposable gloves, facemask, tissue, and protective supplies.
5. Microcentrifuge(s) (with rotor for 1.5 ml and 2 ml centrifuge tubes).

Preparation Before Use

1. If the centrifuge has a refrigeration function, set the temperature to 25℃.
2. Add absolute ethanol to Buffer WA and Buffer WBR according to the instructions on the label of the reagent bottle and tick the box on the label to mark "Ethanol Added".
3. Wear latex gloves and a mask during the entire RNA extraction process, as both saliva and skin contain RNase.
4. To remove DNA from the sample, please add the on-column DNase I digestion step.

Hangzhou Simgen Biotechnology Co., Ltd.
5th floor, Building No. 4, No. 8 Xiyuan 1st Road, Hangzhou, P. R. China.
Pc: 310030 Tel: +86-571-87381295 Fax: +86-571-87381295

Protocol

1. **Add 3 times the sample volume of Buffer L to the sample that needs to be purified, and vortex mix evenly.**

* For example, if a 100 µl sample needs to be purified, 300 µl Buffer L needs to be added.

* If the RNA to be purified is in precipitated form, dissolve the RNA by adding an appropriate amount of RNase- free water (not provided) and adjust the volume to between 100 and 200 µl.

2. **Add 3.2 times the sample volume of absolute ethanol and invert to mix well. Transfer the mixture to a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), close the lid and centrifuge at 12000 rpm for 30 sec.**

* For example, if 100 µl of sample needs to be purified, 320 µl of absolute ethanol needs to be added.

* If you are purifying RNA from a sample larger than 100 µl, centrifuge the mixture through the column in twice.

3. **Discard the filtrate, place the Spin Column back to the 2 ml Collection Tube. Add 700 µl Buffer WA to the Spin Column, close the lid and centrifuge at 12000 rpm for 30 sec.**

* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhering to the mouth of the collection tube, you can slap the 2 ml Collection Tube upside down on a paper towel once.

* Ensure that absolute ethanol has been added to Buffer WA.

4. **Discard the filtrate, place the Spin Column back to the 2 ml Collection Tube. Add 800 µl Buffer WBR to the Spin Column, close the lid and centrifuge at 12000 rpm for 30 sec.**

* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhering to the mouth of the collection tube, you can slap the 2 ml Collection Tube upside down on a paper towel once.

* Ensure that absolute ethanol has been added to Buffer WBR.

5. **Discard the filtrate, place the Spin Column back to the 2 ml Collection Tube and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge does not reach 14,000 rpm, centrifuge at full speed for 2 min.

* Do not omit this step, as this will cause the residual ethanol in the eluate.

6. **Discard 2 ml Collection Tube, place the Spin Column in a clean RNase-free 1.5 ml centrifuge tube, add 50-100 µl RNase-free Water, close the lid, incubate at room temperature for 1 min, centrifuge at 12000 rpm for 30 sec.**

* If the centrifuge does not have a leak-proof cover, cutoff the lid of the 1.5 ml centrifuge to prevent the lid from falling off and damaging the centrifuge.

7. **Discard the Spin Column. The eluted RNA can be used for various molecular biology experiments or stored below -70°C for later use.**