HiPure Serum miRNA Kit

Introduction

The kit offers the unique feature to isolate total RNA including small RNA and DNA from serum and plasma without the need to resort to the cumbersome phenol/chloroform extraction or a time consuming proteinase digest. RNA purified using the kit is ready for applications such as RT-PCR, Northern blotting, poly A+ RNA (mRNA) purification, nuclease protection, and in vitro translation.

Principle

HiPure RNA technology simplifies total RNA isolation. The sample material is denatured in Lysis Buffer. The protein is then precipitated by Protein Precipitation Solution and pelleted by centrifugation. After the removal of protein the binding conditions for nucleic acids are adjusted by adding isopropanol. Total nucleic acids are bound to the column. Optionally, DNA can be removed by an on-column rDNase digest. The remaining nucleic acids are washed and eluted with minimal amounts of RNase-Free water.

Kit Contents

Product	R431402	R431403	Contents
Preparation Times	50	250	—
HiPure RNA Mini Columns	100	2 x 250	Silica Column
2ml Collection Tubes	100	2 x 250	PP Column
Buffer CFL	6 ml	30 ml	Guanidine Salt/Phenol
Buffer CFP	1.8 ml	10 ml	
Buffer RWC*	20 ml	80 ml	Guanidine Salt
Buffer RW2*	20 ml	2 x 50 ml	Tris/EDTA
RNase Free Water	10 ml	60 ml	DEPC-Treated Water

Storage and Stability

The 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

- Dilute Buffer RW2 with 80ml (50 Preps) or 2 x 200ml (250 Preps) 100% ethanol and store at room temperature
- Dilute Buffer RWC with 40ml (50 Preps) or 160ml (250 Preps) 100% ethanol and store at room temperature
- Microcentrifuge capable of at least 12,000 × g

Preparation of plasma from human EDTA blood

1. Centrifuge fresh blood sample for 10 min at 2,000 x g.
2. Remove plasma without disturbing sedimented cells.
3. Freeze plasma at -20 °C for storage upon RNA isolation.
4. Thaw frozen plasma samples prior to RNA isolation and centrifuge for 3 min at ⩾ 11,000 x g in order to remove residual cells, cell debris, and particulate matter. Use the supernatant for RNA isolation.

Protocol

1. Transfer 0.3ml Serum or plasma into 1.5ml microcentrifuge tube.
2. **Add 90µl Buffer CFL to the sample. Mix well and incubate at room temperature for 10min.** To process 600 µL or 900 µL sample material, increase volumes for Buffer CFL, CFP, and isopropanol proportionally. Multiple loading steps will be necessary in step6.
3. **Add 30µL Buffer CFP and vortex for 10 s. Incubate for 1 min at room temperature (18–25 °C).** Centrifuge for 3 min at 11,000 x g to pellet the protein.
4. Transfer the supernatant into a new tube. Add 400 µL isopropanol(2% HAC) and vortex for 5 s.

   For 1ml Isopropanol, add 20ul Glacial acetic acid, mix well.

5. Insert a HiPure RNA Mini Column in a 2ml Collection Tube.

6. **Transfer the mixture to a the column placed in a 2 ml collection tube (supplied).** Incubate at room temperature for 2 min. Centrifuge for 60 s at ⩾12000 x g . Discard the column, and save the flow-through.

   If more than 300 µL plasma / serum was used, repeat this step until all sample is loaded onto the column.

7. **Add 600µl Buffer RWC to the column, Centrifuge at 10,000 × g for 1 minute at room temperature.** Discard the filtrate and reuse collection tube.

2. **Add 500µl Buffer RW2 to the column, Centrifuge at 10,000 × g for 1 minute at room temperature.** Discard the filtrate and reuse collection tube.

3. **Add 500µl Buffer RW2 to the column, Centrifuge at 10,000 × g for 1 minute at room temperature.** Discard the filtrate and reuse collection tube.

4. Centrifuge the empty Column at 10,000 × g for 2 minute at room temperature to dry the column matrix.

5. **Transfer the Column to a clean 1.5ml microcentrifuge tube. Add 30~50µl RNase Free Water directly to the center of the column membrane.** Let sit at room temperature for 2 minutes. Centrifuge at 10,000 × g for 1 minute at room temperature.

6. Store RNA at -20℃.

Troubleshooting Guide

1. Clogged HiPure RNA Column

- **Too much starting material:** In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material.
- **Inefficient disruption and/or homogenization:** Disrupting and homogenizing starting materia as qiagen RNeasy Mini Kit pages 18-21.

2. RNA does not perform well (e.g. in RT-PCR

- **Salt concentration in eluate too high**: Modify the wash step by incubating the column for 5 min at room temperature after adding 500ul of Buffer RW2, then centriufge.
- **Eluate contains residual ethanol:** Ensure that the wash flow-through is drained from the collection tube and that the column is then centrifuged at >12,000 x g for 1min.

3. DNA contamination in downstream expeiments

- **No DNase treatment**: Perform optional on column DNase digestion using RNase-Free DNase Ste at the point individual protocols.

4. Low A260/A280 value

- Water used to dilute RNA for A260/A280 measurement: Use 10 mm Tris·Cl, pH 7.5, not RNAse-free water, to dilute the sample before measuring purity..