HiPure Plant RNA Midi Kit

Introduction

The HiPure Plant RNA Midi Kit provides fast purification of high-quality RNA from Plants, cell, tissues, and yeast using silica-membrane spin columns with a binding capacity of 1mg RNA. There is no need for phenol/chloroform extractions and time-consuming steps such as CsCl gradient ultracentrifugation, or precipitation with isopropanol or LiCl are eliminated. RNA purified using the RaPure Total RNA Purification System is ready for applications such as RT-PCR, Northern blotting, poly A+ RNA (mRNA) purification, nuclease protection, and in vitro translation.

Principle

The Kit isolates total RNA from up to 1g plant tissue. A short workflow enables RNA isolation with genomic DNA removal in less than 25 min. Samples are first lysed and homogenized. The lysate is passed through a DNA Mini column, ethanol is added to the flow-through, and the sample is applied to an RNA column. RNA binds to the membrane and contaminants are washed away. High-quality RNA is eluted in as little as 400 µl water using the Kit.

Kit Contents

Product	R415202	R415103
Preparation Times	20	100
HiPure DNA Midi Column II	20	100
HiPure RNA Midi Columns II	20	100
15ml Collection Tubes	40	200
Buffer RLC	120 ml	550 ml
Buffer PRC1	120 ml	550 ml
Buffer RW1	70 ml	400 ml
Buffer RW2*	20 ml	100 ml
RNase Free Water	30 ml	120 ml

Storage and Stability

The Kit can be stored dry at room temperature (15–25°C) and are stable for at least 18 months under these conditions. Make sure that all buffers are at room temperature when used. During shipment, crystals or precipitation may form in the Buffer RLC/PRC1. Dissolve by warming buffer to 37°C.

Materials and Equipment to be Supplied by User

- Dilute Buffer RW2 with 80ml (20 Preps) or 400ml (100 Preps) 100% ethanol and store at room temperature
- Microcentrifuge capable of at least 12,000 × g
- The Kit provides a choice of lysis buffers. Buffer RLC is the lysis buffer of choice but Buffer RLT can cause solidification of some samples, depending on the amount and type of secondary metabolites in the tissue. In these cases, Buffer PRC1 should be used.
- Add either 10µl β-mercaptoethanol (β-ME), or 20 µl 2 M dithiothreitol (DTT) to 1 ml Buffer RLC or Buffer PRC1 before use. Buffers with DTT or β-ME can be stored at room temperature for up to 1 month.

Protocol

1. **Determine the amount of plant material. Do not use more than 1g.**
   It is essential to use the correct amount of starting material in order to obtain optimal RNA yield and purity. A maximum amount of 1g plant material can generally be processed. For most plant materials, the RNA binding capacity of the RNeasy spin column and the lysing we recommend starting with no more than 500 mg plant material. Depending on RNA yield and purity, it may be possible to use up to 1g plant material in subsequent preparations.

2. **Immediately place the tissue in liquid nitrogen and grind thoroughly with a mortar and pestle. Decant tissue powder and liquid nitrogen into an 15 ml centrifuge tube.** Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw.
   RNA in plant tissues is not protected until the tissues are flash-frozen in liquid nitrogen. Frozen tissues should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

3. **Add 5ml Buffer RLC or Buffer PRC1 to a maximum of 1g tissue powder. Vortex vigorously.** Centrifuge for 10 min at ⩾4,000 x g .
   Vortex or pipet further to remove any clumps. Clumps of tissue will not lyse properly and will therefore result in a lower yield of RNA. A short 3 min incubation at 56°C may help to disrupt the tissue. However, do not incubate samples with a high starch content at elevated temperatures, otherwise swelling of the sample will occur.

4. Insert a HiPure DNA Midi Column in a 15ml Collection Tube.

5. **Transfer 4ml of the supernatant from step 3 to a the DNA Midi column placed in a 15 ml collection tube (supplied).** Centrifuge for 5min at ⩾4,000 x g. Discard the column and save the flow-through.
   Make sure that no liquid remains on the column membrane after centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane.

6. **Add 0.5 volume (usually 2ml) of absolute ethanol to the flow-through, and mix well by pipetting or vortexing.** Do not centrifuge.
   If some lysate was lost during homogenization and DNA removal, adjust the volume of ethanol accordingly.

7. Insert a HiPure RNA Midi Column in a 15 ml Collection Tube.

8. **Add one half of the sample from Step 6 to the Column.**Centrifuge for 5 min at ⩾4,000 x g. Discard the filtrate and reuse collection tube.

9. Repeat Step 8 until all of the sample has been transferred to the column.

10. **Add 3ml Buffer RW1 to the column, centrifuge at ⩾4,000 × g for 3 minute at room temperature.** Discard the filtrate and reuse collection tube.

11. **Add 3ml Buffer RW2 to the column, Centrifuge at ⩾4,000 × g for 3 minute at room temperature.** Discard the filtrate and reuse collection tube.

12. **Add 3ml absolute ethanol to the column, Centrifuge at ⩾4,000 × g for 10 minute at room temperature.** Discard the filtrate and reuse collection tube.

13. **Transfer the Column to a clean 15ml centrifuge tube. Add 400~500µl RNase Free Water directly to the center of the column membrane.** Let sit at room temperature for 3 minutes. Centrifuge at ⩾4,000 x g for 5min.

14. **Repeat step 13 using another volume of RNase-free water, or using the eluate from step 12** (if high RNA concentration is required).

15. 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. If working with tissues rich in proteins, we recommend using the HiPure Fibrous Tissue RNA Mini Kit.

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.
- **Incubation with Buffer RW1：** In subsequent preparations, incubate the RNeasy spin column for 5 min at room temperature after addition of Buffer RW1 and before centrifuging.

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..