

HiPure Fruit RNA Kit

Introduction

The HiPure Fruit RNA Kit provides fast purification of high-quality RNA from Fruits, cell, tissues, and yeast using silica-membrane spin columns with a binding capacity of 100ug 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 HiPure 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 150mg 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 30 µl water using the Kit.

Kit Contents

Product	R401402	R401403
Preparation Times	50	250
HiPure RNA Mini Columns	50	250
2ml Collection Tubes	50	250
PlantZol Reagent	60 ml	200 ml
Buffer GXP	30 ml	150 ml
Buffer RW1	50 ml	200 ml
Buffer RW2*	20 ml	2 x 50 ml
RNase Free Water	10 ml	30 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. 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 (50 Preps) or 2 x 200ml (250 Preps) 100% ethanol and store at room temperature
- Microcentrifuge capable of at least 12,000 x g

Protocol

1. Determine the amount of Fruit material. Do not use more than 150 mg.

It is essential to use the correct amount of starting material in order to obtain optimal RNA yield and purity. A maximum amount of 150 mg Fruit material can generally be processed. For most Fruit materials, we recommend starting with no more than 50 mg Fruit material. Depending on RNA yield and purity, it may be possible to use up to 150 mg Fruit 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 a 2 ml microcentrifuge tube. Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw.

RNA in Fruit 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 1 ml PlantZol Reagent to a maximum of 100 mg tissue powder. Vortex vigorously.

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.

4. Add 0.2 mL of chloroform per 1 mL of PlantZol Reagent and Vortex vigorously for 15 seconds and incubate at room temperature for 3 minutes.

5. Centrifuge the samples at no more than 12,000 x g for 10 minutes 4°C.

6. Transfer 500µl of the supernatant into a new tube and add 500 µl of Buffer GXP. Invert the

tube to mix well.

7. **Add 500 μ l absolute ethanol to the sample and invert the tube to mix well.**
8. Insert a HiPure RNA Mini Column in a 2ml Collection Tube.
9. **Add up to 600 μ l of the sample from Step 7 to the Column.** Centrifuge at 12,000 \times g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.
10. Repeat Step 8 until all of the sample has been transferred to the column.
11. **Add 700 μ l Buffer RW1 to the column, centrifuge at 12,000 \times g for 1 minute at room temperature.** Discard the filtrate and reuse collection tube.
12. **Add 500 μ l Buffer RW2 to the column, Centrifuge at 12,000 \times g for 1 minute at room temperature.** Discard the filtrate and reuse collection tube.
13. **Add 500 μ l Buffer RW2 to the column, Centrifuge at 12,000 \times g for 1 minute at room temperature.** Discard the filtrate and reuse collection tube.
14. Centrifuge the empty Column at 12,000 \times g for 2 minute at room temperature to dry the column matrix.
15. **Transfer the Column to a clean 1.5ml microcentrifuge tube. Add 30~100 μ l RNase Free Water directly to the center of the column membrane.** Let sit at room temperature for 2 minutes.
16. **Repeat step 15 using another volume of RNase-free water, or using the eluate from step 15 (if high RNA concentration is required).**
17. Store RNA at -20°C.

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 centrifuge.
- **Eluate contains residual ethanol:** Ensure that the wash flow-through is drained from the collection tube and that the column is then centrifuged at $>12,000 \times g$ for 1 min.

3. DNA contamination in downstream experiments

- **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 RNA Mini 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..