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. RNA purified using this kit is ready for applications such as RT-PCR, Northern blotting, poly A+ RNA (mRNA) purification, nuclease protection, and in vitro translation.

Kit Contents

Product	R401401	R401402	R401403
Preparation Times	20	50	250
HiPure RNA Mini Columns	20	50	250
2ml Collection Tubes	20	50	2 x 125
PlantZol Reagent	30 ml	60 ml	200 ml
Buffer GXP	150 ml	30 ml	150 ml
Buffer RW1	25 ml	50 ml	200 ml
Buffer RW2*	10 ml	20 ml	2 x 50 ml
RNase Free Water	10 ml	10 ml	30 ml

Storage and Stability

PlantZol Reagent should be store at 2–8°C after received. The remaining components of the kit can be stored dry at room temperature (15–25°C) and are stable for at least 24 months under these conditions.

Materials and Equipment to be Supplied by User

- Dilute Buffer RW2 with 40ml (20 Preps), 80ml (50 Preps) or 2 x 200ml (250 Preps) 100% ethanol and store at room temperature
- Microcentrifuge capable of at least 12,000 × g, 4°C
- Absolute ethanol

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 an 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 1ml Planzol Reagent to the sample and 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 to the sample and Vortex** vigorously for 15 seconds and incubate at room temperature for 3 minutes.

5. **Centrifuge the samples at 12,000 x g for 10 minutes at 4°C.**

   If 4°C centrifuge machine is not available, centrifuge at room temperature.

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 µl 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. **Transfer ⩽ 750µl of the mixture from Step 7 to the Column.** Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.

10. Repeat Step 9 until all of the mixture has been transferred to the column.

11. **Add 700µl Buffer RW1 to the column, centrifuge at 12,000 × g for 1 minute at room**

temperature. Discard the filtrate and reuse collection tube.

Optional: if need to remove DNA thoroughly, customer can proceed with DNase I digestion process by Magen DNase I Set (Cat# C12133, not provided)

12. **Add 500μl Buffer RW2 to the column, Centrifuge at 12,000 × g for 1 minute at room temperature.** Discard the filtrate and reuse collection tube.

13. **Repeat Step 12 once**.

14. Centrifuge the empty Column at 12,000 × g for 2 minute at room temperature to dry the column matrix.

15. **Transfer the Column to a clean 1.5ml microcentrifuge tube. Add 30~100μl 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). Store RNA at -20℃.

The minimum elution volume for the HiPure RNA Column is 30 μl. A second elution is recommended if the RNA yield exceeds 30 μg. This product only recovers RNA larger than 200 nt. RNAs shorter than 200 nt (approximately 15-20 % of total RNA), including 5S RNA, tRNA and others, are removed during purification.

Notes: When total RNA is above 10 μg, the OD260/230 ratio is typically 1.3-2.2. For total RNA ranging from 5 μg to 10 μg, the A260/230 ratio ranges from 0.7-2.0. When total RNA is below 3 μg, the A260/230 ratio may drop below 0.6. This is because Buffer RW1 contains guanidinium isothiocyanate, and the silica membrane exhibits water-absorbing property and background binding. Guanidinium isothiocyanate has strong absorbance at 230 nm. So the A260/230 ratio is mainly affected by this guanidinium salt rather than the sample itself. Research data demonstrate that low levels of residual guanidinium isothiocyanate do not interfere with downstream applications such as reverse transcription, quantitative RT-PCR and next-generation sequencing. Ratios of 0.2-1.0 for A260/230 can be disregarded, and the RNA may be directly used for downstream assays including reverse transcription. By condition of no column clogging occurs, increasing the input sample amount and lysis-buffer volume appropriately to raise total nucleic-acid yield (>10 μg) can substantially improve the OD260/230 ratio.

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 material. If working with tissues rich in proteins, we recommend using the HiPure Plant RNA Plus Kit (Magen #R415002).

2. Low yield or degradation of isolated RNA

- **Electrophoresis-related issues:** RNA degradation frequently occurs during electrophoresis. Replace with fresh Loading Buffer and electrophoresis buffer.
- **Contaminated RNase-Free Water:** RNase-Free Water contains no bacteriostatic agents. Bacterial or fungal contamination may be introduced upon storage at room temperature or during handling. Use fresh RNase-Free Water or DEPC-treated water.
- **Insufficient elution:** Apply RNase-Free Water directly onto the membrane. Incubate for several minutes before centrifugation. Perform a second elution to improve yield..

3. DNA contamination in downstream expeiments

- **Excessive transfer of supernatant:** Transfer only 400-450 µl of supernatant in step 6, the interphase is rich in DNA.
- **No DNase treatment**: Perform optional DNase I digestion process in Step 12.

4. Poor purity (OD260/280 < 1.65)

- Failure to vigorously vortex after adding Buffer BCP. Do not mix by vortexing or inverting the tube
- Over-addition of Buffer BCP.
- Incomplete lysis: Incubate the homogenate at room temperature for 5 min after homogenization
- Reduce sample input