imaGen

ImaSpin® Total RNA Extraction Kit –Plant Plus

For total RNA extraction from **ployphenol- and polysaccharide-rich** Plant

Precautions

I. Handling Requirements
When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

II. Equipment and Reagents to Be Supplied by User

- Ethanol (96–100 %)*
- 1.5 ml microcentrifuge tubes
- Pipet tips with aerosol barrier
- Vortexer
- Microcentrifuge (with rotor for 1.5 ml tubes) may be required for some samples
- ß - Mercaptoethanol (ß - ME)
- DNase I (optional)

*Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

III. Waste Handling

- Treat waste with the country, federal, state and local regulations.

IV. Important points before use

- Do not use the product if it has expired.
- Add absolute ethanol (see the bottle label for volume) to IRW2 Buffer then mix by shaking for a few seconds and tick the checkbox of the label on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Kit Contents

IRPP Buffer
IRPW1 Buffer
IRW2 Buffer (Add Ethanol)
RNase-free Water
IR Column
2 ml Collection Tubes

Storage and Stability:

This kit should be stored at room temperature.

Description

ImaSpin® Total RNA Extraction Kit –Plant Plus is designed by patented technology for purification of total RNA from ployphenol- and polysaccharide-rich plant tissue and cells. The method uses detergents and a chaotropic salt to lyse the cells and inactivate RNase. RNA in chaotropic salt solutions binds to the glass fiber matrix of the IR columns. Following washing off of contaminants, the purified RNA is eluted by RNase-free water. ssRNA and dsRNA can be efficiently purified. Purified RNA is ready for RT-PCR, northern blotting, primer extension and cDNA library construction.

ImaSpin® Total RNA Extraction Kit –Plant Plus Test Data

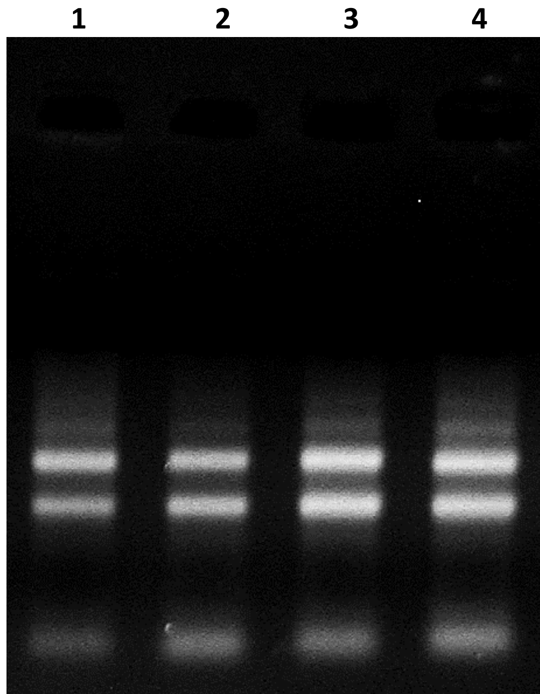

Fig 1. Total RNA extracted from fresh Mango fruit tissue

Total RNA from 100 mg Mango (*Mangifera indica*) fresh fruit tissue was extracted using the ImaSpin® Total RNA Extraction Kit –Plant Plus & Competitive brand Q. 10 µl from 100 µl eluates of purified Total RNA was analyzed by electrophoresis on a 1 % agarose gel.

1-2 = Competitive brand Q

3-4 = ImaSpin® Total RNA Extraction Kit –Plant Plus

Preparation before using

1. Total RNA Extraction Kit has been optimized for preferential RNA binding, however genomic DNA contamination is almost impossible to avoid during RNA extraction procedures. DNase I (RNase-Free) may be applied to the binding column according to the protocol instructions. It is necessary to use highly purified DNase. If RNase is present in trace amounts it will result in RNA degradation. It is recommended to apply DNase for sensitive downstream applications, however for many downstream applications it may not be necessary to apply as genomic DNA contamination may be negligible or inconsequential to the application.
2. Wear a lab coat and disposable gloves to prevent RNase contamination.
3. Before use, add 10 µl of ß-ME to 1 mL of IRPP Buffer. IRPP Buffer Containing ß-ME can be stored at room temperature for up to 1 month.
4. Prepare 70 % EtOH in advance.

Protocol

1. Cut off 50 mg (up to 100 mg) of fresh or frozen plant tissue.
2. Grind the sample under liquid nitrogen to a fine powder.
3. Transfer it into a microcentrifuge tube (not provided). Certain plant samples may not require liquid nitrogen treatment.
4. Add 400 µl of IRPP Buffer (ß-ME added) into the tube and vortaxing to mix well for 10 seconds.
5. Incubate at room temperature for 5 minutes.
6. If insoluble material remains following incubation, centrifuge for 2 minutes at 13,000 rpm (10,000 x g) and transfer all the clarified supernatant to a new microcentrifuge tube (not provided).
7. Add 400 ul of 70% EtOH to the sample lysate and mix by pipetting immediately for 10 seconds.
8. Place a IR Column in a 2 ml Collection Tube. Transfer all of the mixture to the IR Column.
9. Centrifuge at 13,000 rpm (10,000 x g) for 30 seconds.
10. Discard the flow-through then place the IR Column back in the 2 ml Collection Tube.
    Optional: If performing optional DNase digestion
11. Add 600 µl of IRPW1 Buffer to the IR Column.
12. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute, then discard the flow-through. Place the IR Column back in the 2 ml Collection Tube.
13. Add 600 µl of IRW2 Buffer (make sure absolute ethanol was added) to the IR Column.
14. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute then discard the flow-through. Place the IR Column back in the 2 ml Collection Tube.
15. Add 600 µl of IRW2 Buffer (make sure absolute ethanol was added) to the IR Column.
16. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute then discard the flow-through. Place the IR Column back in the 2 ml Collection Tube.
17. Centrifuge for 3 minutes at13,000 rpm (10,000 x g) to dry the column matrix.
18. Transfer the dried IR Column to a clean 1.5 ml microcentrifuge tube.
19. Add 50 µl of RNase-free Water into the CENTER of the column matrix.
20. Let stand for at least 3 minutes to allow RNase-free Water to be completely absorbed.
21. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute to elute the purified RNA.

Optional Step:

DNA residue degradation Procedure

1. Add 200 µl of Buffer IRPW1 Buffer to the IR column. Close the lid gently, and centrifuge for 15 seconds at 13,000 rpm (10,000 x g) to wash the spin column membrane. Discard the flow-through.
2. Add 10 µl of DNase I stock solution (3 Kunitz units / µl) to 70 µl of Buffer DNase I Reaction Buffer. Mix by gently inverting the tube, and centrifuge briefly to collect residual liquid from the sides of the tube.
   Note: DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube. Do not vortex.
3. Add the DNase I mixture (80 µl) directly to sample tube, and incubate at 20 – 30 °C for 15 minutes.
4. Add 200 µl of Buffer IRPW1 Buffer. Close the lid gently, and centrifuge for 15 seconds at 13,000 rpm (10,000 x g) to wash the spin column membrane. Discard the flow-through.
5. Continue with the IRPW1 Buffer wash step.

Troubleshooting

Problem	Possible Reasons/Solution
DNA contamination	➢ Perform in Column DNase I Digestion to eliminate DNA contamination.
Eluted RNA does not perform well in downstream applications	➢ Residual Ethanol Contamination: Following the wash step, dry the IR Column with additional centrifugation at 13,000 rpm (10,000 x g) for 5 minutes.
Low RNA Yield	➢ Ensure absolute ethanol was added to IW2 Buffer and close the bottle tightly after each use to avoid ethanol evaporation. Insufficient disruption and homogenization/too much starting material, try to adjust it. ➢ RNA still bound to the IR Column membrane, elute twice to increase the yield. ➢ Ethanol carryover; following the wash step, dry the IR Column with additional centrifugation at 13,000 rpm (10,000 x g) for 5 minutes. ➢ Ensure RNase-free Water is added into the CENTER of the column matrix.
RNA Degradation	➢ Harvested sample immediately stabilized/inappropriate handling of starting material. ➢ Avoid RNase contamination by always wear gloves & mask and treat all the equipment with RNaseOUT.