

PURESCRIPT® RNA Purification Kit

Total RNA Purification From 200 mg Plant Tissue

Expected Yield Range: 200-400 µg RNA

Cell Lysis

1. Add 200 mg (0.2 g) frozen ground tissue or fresh tissue to a tissue grinder tube on ice and then add 6 ml **Cell Lysis Solution**. Grind frozen tissue finely in liquid nitrogen with a porcelain mortar and pestle. Keep tissue frozen until added to the tissue grinder tube.
2. Homogenize quickly using 5-10 strokes with a tube pestle. Transfer sample to an Oak Ridge centrifuge tube (or other tube rated for high speed).

Protein-DNA Precipitation

1. Add 2 ml **Protein-DNA Precipitation Solution** to the cell lysate.
2. Invert tube gently 10 times and place tube into an ice bath for 10 minutes.
3. Centrifuge at 15,000 x g for 5 minutes. The precipitated proteins and DNA will form a tight pellet.

RNA Precipitation

1. Pour the supernatant containing the RNA (leaving behind the precipitated protein-DNA pellet) into a clean Oak Ridge centrifuge tube (or other tube rated for high speed) containing 6 ml **100% Isopropanol** (2-propanol).
2. Mix the sample by inverting gently 50 times.
3. Centrifuge at 15,000 x g for 5 minutes; the RNA will be visible as a small, translucent pellet.
4. Pour off supernatant and drain tube on clean absorbent paper. Add 6 ml **70% Ethanol**. Invert the tube several times to wash the RNA pellet.
5. Centrifuge at 15,000 x g for 2 minutes. Carefully pour off the ethanol.
6. Invert and drain the tube on clean absorbent paper and allow to air dry 15 minutes.

RNA Hydration

1. Add 600 µl **RNA Hydration Solution** (600 µl will give a concentration of 500 µg/ml if the total yield is 300 µg RNA).
2. Allow RNA to rehydrate at least 30 minutes on ice. Vortex sample vigorously for 5 seconds, pulse spin and carefully transfer to a 1.5 ml microfuge tube. Store at -70°C to -80°C.
3. Before use, vortex sample vigorously for 5 seconds and pulse spin. Pipet sample up and down several times to ensure adequate mixing.
4. Store purified RNA sample at -70° to -80°C.