Cyrusbioscience

# GeneKlean® Total RNA Isolation Kit-Plant

**Cat. Number:** TRNAP

**Storage:** Store at Room Temperature.

**Sample:** 100mg of fresh plant tissue or 25mg of dry plant tissue

**Yield:** Up to 30ug Total RNA

## Kit Contents

| Total RNA Isolation Kit-Plant | 100 Reactions |
|---|---|
| GeneKlean® Total RNA Column with 2 mL Collection Tube | 100 |
| Lysis Buffer | 110mL |
| Wash Buffer 1 | 45mL |
| Wash Buffer 2 | 15mL |
| (add Ethanol) | (60mL) |
| Elution Buffer | 10mL |
| This Datasheet | 1 copy |

**Note**

- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination.
- Add ethanol (>99.5%) to Wash Buffer 2, shake before use (see bottle label for volume).
- Check Buffers before use for salt precipitation. Redissolve any precipitate by warming to 37°C.
- Buffers contain irritants. Wear gloves when handling these buffers.

## Additional requirements

- Ethanol (>99.5%)
- Isopropanol
- RNase-free pipet tips and 1.5 ml microcentrifuge tubes
- b-mercaptoethanol

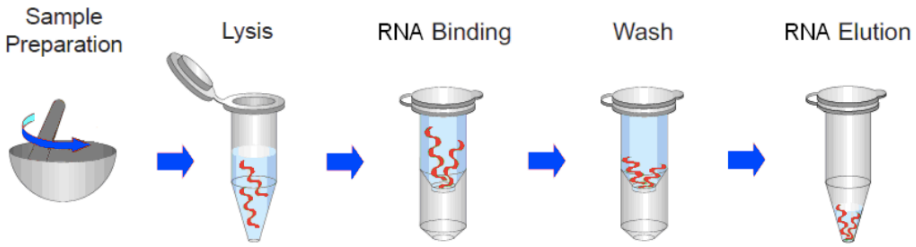
## Optional Step for DNA Degradation:

Add 2 µl DNAse I (2KU/ml) and 10 µL reaction buffer (300 mM Tris-HCl (pH 7.5), 60 mM MnCl2, 300 µg/ml BSA) to the 50µl final product. Let stand for 10 minutes at room temperature (at 25°C).

## Description

GeneKlean® Total RNA Isolation Kit-Plant provides a fast, simple, and cost-effective method for isolation of total RNA from plant samples. Detergents and chaotropic salt are used to lyse cells and inactivate RNase. The specialized high-salt buffering system allows RNA species longer than 100 bases to bind to the the glass fiber matrix of the spin column . GeneKlean® Total RNA Isolation Kit-Plant is suitable for a variety of routine applications including RT-PCR, cDNA Synthesis, Northern Blotting, Differential display, Primer Extension and mRNA Selection. The entire procedure can be completed within 60 minutes.

**Procedure for Preparation of Plant Total RNA Isolation**




**Sample Preparation**

1. Cut off 100 mg of fresh plant tissue or 50 mg of dry plant tissue.
2. Grind the sample under liquid nitrogen to a fine powder using a mortar and pestle.

**Step 1. Lysis**

1. Add 1mL of Lysis Buffer and 10uL of b-Mercaptoethanol to the sample in the motor and grind the sample until it is dissolved completely.
2. Transfer the dissolved sample to RNase-free 1.5mL microtube. Incubate at 70°C for 30 mins.(Invert the tube every 10 mins)
3. Centrifuge at 4°C at 15,000 x g for 10 mins. Transfer the supernatant to a new 1.5mL microtube.

**Step 2. RNA Binding**

1. Add 1/2 volume of Isopropanol to the sample from Step 1 and shake vigorously (eg. 250uL of Isopropanol to 500uL of sample)
2. Place GeneKlean® Total RNA Column in a 2mL Collection Tube.
3. Transfer the sample mixture to the GeneKlean® Total RNA Column. Centrifuge at 15,000x g for 30 secs.
4. Discard the flow-through and transfer the remaining mixture to the same GeneKlean® Total RNA Column.
5. Centrifuge at 15,000x g for 30 secs.
6. Discard the flow-through and place the GeneKlean® Total RNA Column back to the 2mL Collection Tube.

**Step 3. Washing**

1. Add 400uL of Wash Buffer 1 to the GeneKlean® Total RNA Column. Centrifuge at 15,000x g for 30 secs.
2. Discard the flow-through and place the GeneKlean® Total RNA Column back to the 2mL Collection Tube.
3. Add 600uL of Wash Buffer 2(ethanol added) into GeneKlean® Total RNA Column. Centrifuge at 15,000x g for 30 secs.
4. Discard the flow-through and place the GeneKlean® Total RNA Column back to the 2mL Collection Tube.
5. Centrifuge again for 3 mins at 15,000x g to dry the column matrix.

**Step 4. Elution**

1. Place GeneKlean® Total RNA Column. Centrifuge in a clean RNase Free 1.5mL microtube.
2. Add 50-200uL of Elution Buffer to the center of GeneKlean® Total RNA Column, stand for 2 mins. Centrifuge 2 mins at 15,000x g

## Troubleshooting

| Problems | Possible Causes | Suggestions |
|---|---|---|
| Degraded RNA/Low integrity | RNase Contamination | Clean everything, use barrier tips, wear gloves and a lab coat, and use RNase-free enzymes, Ex: RNase inhibitor. |
| Low yields of RNA | Incomplete lysis and homogenization | Don’t use more samples than the suggested limit. |
| | Incorrect elution conditions | Add 100 uL of the Elution Buffer to the center of each Column, let it stand for 2 minutes, and centrifuge at 15,000 x g for 2 minutes. |
| Inhibition of downstream enzymatic reactions | Presence of ethanol in the purified RNA | Repeat the wash step: Centrifuge at 15,000 x g again for 2 minutes to remove the residual of Wash Buffer 2. |