Quick-Start Protocol

CarbonPrep Trizol/Phenol

Upon receiving your kit

- Add molecular biology grade ethanol to each bottle which requires it. Check the boxes when this is complete.
- **Optional:** You may choose to dilute the RPS V buffer 3:4 depending on your sample. In general, RPS should be diluted if the source sample has high salt content, like cultured cells. If unsure, perform an extraction and run RNA electrophoresis. If DNA contamination is present dilute the RPS V buffer by adding 50 μL of water for every 150 μL of RPS B Buffer (3:4) or by adding 10 ml of RNase free water to a new and unused bottle.
- Neutralization buffer should be stored at 4 ºC long term but is stable at RT for at least 1 month.

Preparing for extractions

- Take 70 μL of carbon beads, place them in a magnetic stand. After the beads have separated, remove the clear storage buffer. You will use the beads in step 4.
- Dilute neutralization buffer 1:10 with molecular biology grade water. You will use 100 μL per reaction in step 6. The elution buffer is molecular biology grade water and can be used for dilution.
- In all mix steps, do at least 6 pipette strokes for best results.
- If using cultured cells, carefully remove all cell wash buffer from pellet before starting. Cell wash buffer is PBS and PBS reduces RNA yield with every RNA isolation kit we've tested! Remove as much PBS as possible.

Procedure

1) **Lysis:** add 200 μL of Trizol to a cell pellet containing 2 to 5 million cells or 2 to 10 mg of tissue. Refer to table 1 if the sample input falls outside of this range.
2) Add 7 μL of RPS V buffer.

(**Optional:** if working with chloroform, get the aqueous layer and start from step 3. This is generally only required or recommended if the source sample is a difficult plant tissue.)

3) Lyse the sample by syringe, pipetting up and down, or vortexing. Homogenize the sample until it is no longer viscous. Incubate for 3 min at room temperature. You may use any homogenization method such as bead

beating, sonication, spinning, ect. as long as sample is homogenized. Viscous lysate will result in DNA becoming physically trapped. Therefore, consider modifying the homogenization method if DNA contamination is observed.

4) Transfer the solution to the tube containing the carbon beads. Mix well by pipetting up and down. Incubate for 1 min.
5) **Binding:** Add 200 μL of binding buffer and mix well immediately. The texture of the carbon beads will change. This is RNA binding to the beads. Mix immediately after adding ethanol to avoid creating pockets of high ethanol concentration that denature DNA and lead to DNA binding.
6) Add 100 μL of neutralization buffer prepared in setup and mix well. This helps release any trapped DNA.
7) **Washing:** Add 750 μL of RPS Wash Buffer (WBRPS) and mix well. Place on a magnetic stand for 45 secs or until black aggregates are collected, and then remove supernatant.
8) Add 750 μL of Wash Buffer (WB) and mix well. Place on a magnetic stand for 45 secs or until black aggregates are collected, and then remove supernatant.
9) Add 500 μL of Wash Buffer (WB) and mix well. Place on a magnetic stand for 45 secs or until black aggregates are collected, and then remove supernatant. For best results, transfer the mixture to a fresh 1.5 ml tube.
10) Remove all liquid and then dry the beads for 10 mins (will dry faster if placed in a hood). After a few minutes, remove liquid again; residual liquid on walls may pool at the bottom of the vial. Ethanol promotes binding so dry beads long enough such that the ethanol evaporates but not so long such that the water also evaporates.
11) After drying add 30 - 100 μL of elution buffer, 50 μL is recommended. Heat the sample at 65 °C for 5 min to fully elute RNA.
12) After heating, mix/flick samples. Then centrifuge at 10,000 g for 2 min to separate beads from RNA elute. Alternatively, place the tube on a magnetic stand and wait 10 minutes for the beads to fully separate.
13) Place tube on magnetic stand and collect the clear liquid which contains pure RNA.

Reagent Volume Quick Reference

Input Tissue	Input Cells	Trizol Volume	RPS V	Carbon Beads	Binding Buffer
< 2 mg	<1 Million	100 µL	3.5 µL	40 µL	100 µL
2-10 mg	2M to 5M	200 µL	7 µL	70 µL	200 µL
>10 mg	>5 Million	300 µL	10.5 µL	70 µL	300 µL

***Reagents not listed, including neutralization buffer, wash buffers, and elution buffers do not change volume based on input material.**