Quick-Start Protocol

CarbonPrep Trizol/Phenol - Plant

READ FIRST: This is a modification of the standard Trizol/Phenol protocol intended for difficult plant tissues. Acid guanidinium-phenol lysis buffers are the most robust and consistent lysis buffers available, but some plant tissues may still be challenging. This protocol is only recommended for difficult plant samples where the standard Trizol/Phenol protocol is not working. The main difference is the inclusion of the chloroform step to deplete polysaccharides from the aqueous layer.

Upon receiving your kit

- Add molecular biology grade ethanol to each bottle that requires it. Check the boxes when this is complete. **Note:** this protocol will not use "binding buffer." That bottle does not need to be filled if you will only be using this protocol.
- **Optional:** You may choose to dilute the RPS B buffer 3:4 depending on your sample. It's unlikely that plant samples will require this. See the main Trizol/Phenol protocol for details.
- Neutralization buffer should be stored at 4 °C long term but is stable at room temperature for at least 3 months.

Preparing for extractions

- Follow the standard Trizol procedure until the aqueous layer is collected after mixing with chloroform, the chloroform will remove polysaccharides. Use the aqueous layer in the protocol below.
- 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 2.
- In all mix steps, do at least 6 pipette strokes for best results.

Procedure

1) Add 300 μL of RPS B buffer to 300 μL of aqueous phase. Incubate for 4 minutes. This protocol assumes 300 μL of aqueous phase. For other volumes, see Table 1.
2) Transfer the solution to the tube containing the carbon beads. Mix well by pipetting up and down. Incubate for 1 min.
3) **Binding:** Add 450 μL of ethanol and mix well immediately. The texture of the carbon beads will change. This is RNA binding to the beads.
4) **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.

5) 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.
6) 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.
7) 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.
8) 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.
9) 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.
10) Place tube on magnetic stand and collect the clear liquid which contains pure RNA.

Reagent Volume Quick Reference

	H_2O Phase	RPS B	Carbon Beads	Ethanol
Example*	300 µL	300 µL	70 µL	450 µL
Ratio**	1	1	0.23	1.5

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

**** The example protocol assumes 300 µL aqueous phase as input, for other inputs, scale reagents according to the ratios in the table. For example, if using 200 µL of aqueous phase, use (1 * 200 µL) = 200 µL of RPS B and (0.23 * 200 µL) = 46 µL of Carbon Beads.**