Quick-Start Protocol

CarbonPrep Trizol/Phenol - Plant

Shelf-life

- Carbon beads have a shelf-life of at least two years.
- RNA pre-binding solution (RPS), RPS wash buffer (RPSWB) and wash buffer (WB) have a shelf life of one year.

Further Information

- Full manual: coming soon.
- Safety Data Sheets: See product page.
- Technical assistance: support@magnetics.life

Before Starting

- Add ethanol to bottles which require it. The amount to add is listed on the bottle. After adding ethanol, mark the bottle in the space provided on the label.
- The complete kit will have seven solutions, RPS, Wash buffer (WB), RPS Wash buffer (WB), Carbon beads, and elution buffer (EB).
- Add 70 µL carbon-beads to a tube, apply a magnet and remove the liquid after 2 mins (use this in step 3)

Procedure

1) Reagent volumes in this protocol are based off of the amount of starting TRIzol. The recommended volume is 250 µL of Trizol for 50 mg of plant tissues. If you wish to use other volumes see the table below to adjust the protocol accordingly.

   Add 250 µL of Trizol and lyse the sample using a syringe and/or pipetting up and down. Difficult tissues like plant tissues may require you to heat the samples at 65 C for up to 20 min with periodic vortex after every 10 min for 10-15 seconds. Work to lyse the sample until it is no longer viscous.

Optional: If you are using the layer separation protocol with chloroform, perform the procedure as normal and when you've collected the aqueous layer continue with step 2.

2) Let samples cool down at RT and then add 250 µL of RPS buffer (equal volume) and mix thoroughly and incubate for 4 mins at RT.
3) Transfer the above solution to the carbon-bead tube and mix and incubate for 1 min at RT.
4) Add 375 µL of pure ethanol to the above solution (1.5x the volume of the aqueous solution/TRIzol reagent). Mix well by pipetting up and down at least 6 times, pipette up and down at least 6 times in every step below that requires "mixing well." Place

on a magnetic stand for 45 secs or until black aggregates are collected, and then remove supernatant

5) Wash the beads by adding 750 µL of RPS wash buffer (RPSWB) and mixing well. Place on a magnetic stand for 45 secs or until black aggregates are collected, and then remove supernatant.

6) Wash the beads two more times with wash buffer (WB). Wash the first time by adding 500 µL of wash buffer and mixing well. Transfer the suspension to a fresh Eppendorf tube to ensure all impurities are removed, then place on a magnetic stand for 45 secs or until black aggregates are collected and remove the supernatant. Wash again by adding 500 µL of wash buffer and mixing well. Place the vial on the magnetic stand and wait until the black aggregates are collected then aspirate out the supernatant.

7) Dry the bead for 10 mins (will dry faster if placed in a hood)

8) After drying add 30 - 100 µL of elution buffer, 50 µL is recommended, mix and heat samples for 5 min at 65 degree to promote RNA release.

9) After heating, mix/flick samples. Then centrifuge at 10,000 g for 2 min to separate beads from RNA elute.

10) After centrifugation step, place tube on magnetic stand and collect the clear liquid which contains pure RNA.

Reagent Volume Quick Reference

	H_2O Phase	RPS	Carbon Beads	Ethanol	RPSWB	WB	Elution
Example*	250 µL	250 µL	70 µL	375 µL	750 µL	500 µL	30-100 µL
Ratio**	1	1	0.28	1.5	750 µL	500 µL	30-100 µL

***250 uL of Trizol as an example.**
****For other amounts of tissue and aqueous phase, use the table. RPS, Carbon beads, and RBS volumes should be scaled to the amount of aqueous phase.**