Quick-Start Protocol

CarbonPrep Trizol/Phenol

Shelf-life

- Carbon beads have a shelf-life of at least two years.
- RNA pre-binding solution (RPS B), RPS wash buffer (RPSWB), wash buffer (WB) and Binding buffer (BB) 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 five solutions, RPS B, Wash buffer (WB), Carbon beads, BB, and elution buffer (EB).

Procedure

1) Lysis:
    a. Add 150 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.
    b. Homogenize the sample until it is no longer viscous. viscosity will promote DNA binding during the binding step.
        i. You can homogenize the sample through pipetting or vortexing.
    c. Add 150uL of RPS B and homogenize
    d. Incubate at room temperature for 10 minutes.
2) While the sample is incubating, add 50-75uL of magnetic beads to a fresh 1.5mL microcentrifuge tube. Place on the magnetic stand and remove the storage buffer after the beads have fully separated on the stand.
3) After the incubation is complete, add the homogenized sample to the magnetic beads, mix 3 times, close the cap and set in a rack at room temperature for 10 minutes.
    a. It is important not to over mix at this phase or there may be sticking of beads to the pipette tip.
4) Add 300 µL of binding buffer to the above solution. Mix well by pipetting up and down at least 6 times.

a. For the best results, pipette up and down vigorously at least 6-8 times in every step below that requires "mixing well."

5) Place on a magnetic stand for 90 secs and then remove supernatant.
6) 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.
7) Wash the beads once with 750uL wash buffer (WB) and mix well. 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. Move the solution to a fresh 1.5mL microcentrifuge tube to ensure any lingering impurities will be removed. Place the vial on the magnetic stand and wait until the black aggregates are collected then aspirate out the supernatant.
8) Dry the bead for 10 mins (will dry faster if placed in a hood)
9) Remove any remaining ethanol and dry for another 10 minutes.
10) 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.
11) After heating, mix/flick samples. Then centrifuge at 10,000 g for 2 min to separate beads from RNA elute.
12) After the centrifugation step, place the tube on a magnetic stand and collect the clear liquid which contains pure RNA.

Reagent Volume Quick Reference

	Tissue	H2O Phase	RPS B	Carbon Beads	BB	RPSWB	WB	Elution
Example*	5 mg	150 µL	150 µL	70 µL	300 µL	750 µL	750/500 µL	30-100 µL
Ratio**	< 1 mg	1	0.5	0.46	1	750 µL	750/500 µL	30-100 µL
	1-10 mg	1	0.5	0.46	1	750 µL	750/500 µL	30-100 µL
	10-20 mg	1	0.5	0.46	1	750 µL	750/500 µL	30-100 µL

***The above protocol uses 5 mg of tissue and 150 uL of aqueous phase as an example.**
****For other amounts of tissue and aqueous phase, use the table. RPS B and Carbon beads volumes should be scaled to the amount of aqueous phase obtained. For example, if 200 µL of aqueous phase is obtained, use 32 µL of RPS.**

FAQs

1. Lysis is the most important step of the extraction protocol. If you are seeing large amounts of DNA yield check your lysis step. Ensure there is no viscosity before adding in the RPS B.
2. If you are noticing a fluffing to your beads (appears to grow in size) after the extraction is complete, there are two things to check
    a. Is there a lot of DNA in your sample? If there is, this can be attributed to the fluffiness of the beads.
        i. Adjust your lysing step by either mixing the sample more, vortexing or sonicating to eliminate DNA contamination.
    b. Are you using the right concentration of RPS B? Too much of the RPS B can cause the beads to fluff up. Ensure you are adding in the proper volumes at the proper concentrations.
3. Are you experiencing lower yield than expected?
    a. Make sure that during your mixing steps, you are mixing vigorously and enough strokes. The mixing breaks up the beads and allows the release of the CaCl2 which in turn aids in the release of RNA in the elution buffer.
    b. Ensure you are using RNase Free reagents, consumables and tips. Any RNase activity can degrade your samples RNA causing lower-than-expected results.