Quick-Start Protocol

CarbonPrep Cultured Cells

Shelf-life

- Carbon beads have a shelf-life of at least two years.
- RNA lysis buffer (Lysis Buffer Cells), RPS (pre-binding agent), 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 seven solutions, lysis buffer, carbon-beads, binding buffer, wash buffer (WB), RPS wash buffer (RPSWB), RPS, and elution buffer.
- Proteinase K (10 mg/ml) is recommended in the protocol, but not provided.

Procedure

- Reagent volumes are based on the number of cells. This protocol assumes 3 million cells. For other amounts see the table below. If purifying from cell lines rich in ribonucleases, you can optionally add 2 µL of β-mercaptoethanol (BME) to each sample.
- Add 150 µl of lysis buffer and (if choosing to use it) 10 ul of Proteinase K (10 mg/ml) to the sample (If not Trizol). Lyse the cells by using a syringe and/or pipetting up and down and/or vortex the pellet. Homogenize the cells thoroughly and make sure lysate is not viscous. (Note: If the lysate is too viscous, incubate at room temperature for a few minutes and homogenize again).
    a. Lysis is the most important step in the protocol. For best results, ensure the sample is fully lysed before moving onto the next step.
- Add lysed sample onto 70 µL of dry carbon beads directly and mix well by pipetting up and down. (makes an even dark solution)
- Allow the sample to incubate at room temperature with the beads for up to 10 minutes.
- Add 250 µl of binding buffer directly to the lysate-bead solution and mix well by pipetting up and down. (Note: You should see flocculation if it's working)

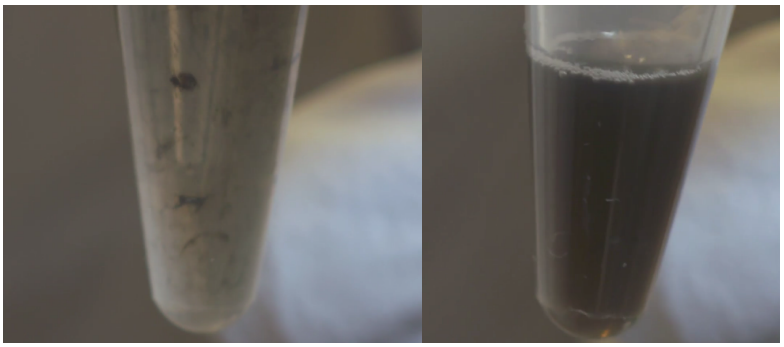

Figure 1: Picture of floccule formation after step 4 using cultured cells as the source sample.

- Place the tubes on the magnetic stand for 90 seconds or until the beads have separated and aspirate out the supernatant.
- Add 750 µL of RPS wash buffer and mix well to wash the beads. Place on a magnetic stand for 45 secs or until black aggregates are collected, and then remove supernatant.
- Wash again by adding 750 µL of wash buffer and mixing well. Then place on a magnetic stand for 45 secs or until black aggregates are collected and remove the supernatant.
- Wash the beads again by adding 500 µL of wash buffer and mixing well. Optionally, transfer the suspension to a fresh Eppendorf tube to ensure all impurities are removed. Place the vial on the magnetic stand and wait until the black aggregates are collected then aspirate out the supernatant.
- Dry the bead for 10 mins, use a p200 to aspirate any remaining ethanol and dry for another 5 minutes (they will dry faster if placed in a hood). Ethanol promotes binding, allow all the ethanol to evaporate before eluting.
- 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, flicking occasionally.
- After heating, mix/flick samples. Then centrifuge at 10,000 g for 2 min to separate beads from RNA elute.
- After the centrifugation step, place the tube on a magnetic stand and collect the clear liquid which contains pure RNA.

Reagent Volume Quick Reference

	Millions of Cells	Lysis Buffer	Carbon Beads	BB	NB	WB	Elution
Example*	3 M	150 µL	70 µL	250 µL	100 µL	750/500 µL	30-100 µL
Ratio**	<2 M	125 µL	60 µL	210 µL	50 µL	750/500 µL	30-100 µL
	2 to 5 M	150 µL	70 µL	250 µL	100 µL	750/500 µL	30-100 µL
	>5 M	200 µL	90 µL	330 µL	150 µL	750/500 µL	30-100 µL

***The above protocol uses 3 million cells as an example. For other amounts, use the values in the table.**

****For 50,000 cells or less it may be desirable to scale the reaction volumes proportionally.**

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 the lysate to the dry beads.
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. (For Trizol) 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.