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), wash buffer (WB), and binding buffer (BB) have a shelf life of one year.
- Neutralization buffer stock solution (NB) has a shelf-life of six month when kept at 4 degrees. Neutralization buffer solution is 1M citric acid. If it is suspected that the NB solution has gone bad, this component can be re-ordered individually or can be prepared in the lab using RNase free water and citric acid.

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.
- Dilute neutralization buffer. For every 10 µl of neutralization buffer add 90 µl of RNase free water to create a 0.1M citric acid solution.
- The complete kit will have six solutions, lysis buffer, carbon-beads, binding buffer, neutralization buffer, wash buffer (WB), and elution buffer.

Procedure

1) Reagent volumes are based off of the number of cells. This protocol assumes 3 million cells. For other amounts see the table below. Completely remove cell wash buffer before starting. It has been shown that leaving 20 to 30 µL of PBS can reduce RNA yield by up to 30% using both this kit and other popular commercial kits. If purifying from cell lines rich in ribonucleases, you can optionally add 2 µL of β-mercaptoethanol (BME) to each sample.
2) Add 150 µl of lysis buffer to the sample. Lyse the cells by using 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 few minutes and homogenize again).
3) Add 70 µL of carbon beads onto the cell lysate directly and mix well by pipetting up and down. (makes an even dark solution)
4) 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)

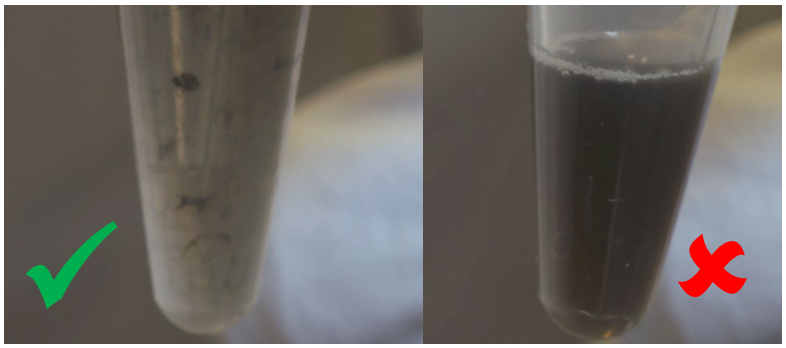

Figure 1: Picture of floccule formation after step 4 using cultured cells as the source sample.

5) Add 100 µl of diluted neutralization buffer solution and mix well.
6) Place the tubes on the magnetic stand for 90 seconds and aspirate out the supernatant.
7) Wash the beads by adding 750 µL of wash buffer and mixing well. Place on a magnetic stand for 45 secs or until black aggregates are collected, and then remove supernatant.
8) Wash again by adding 750 µL of wash buffer and mixing well. Optionally 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.
9) Wash the beads 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.
10) Dry the bead for 10 mins (they will dry faster if placed in a hood). Ethanol promotes binding, allow all the ethanol to evaporate before eluting.
11) 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.
12) After heating, mix/flick samples. Then centrifuge at 10,000 g for 2 min to separate beads from RNA elute.
13) After centrifugation step, place tube on 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**	<1 M	125 µL	60 µL	210 µL	50 µL	750/500 µL	30-100 µL
	1 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.**