Quick-Start Protocol

CarbonPrep Trizol/Phenol

Shelf-life

- Carbon beads have a shelf-life of at least two years.
- RNA pre-binding solution (RPS), RPS wash buffer (RPSWB), 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, possible solutions include remaking it in the lab or reordering online.

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 ul of neutralization buffer add 90 ul of RNase free water to create a 0.1M citric acid solution.
- The complete kit will have seven solutions, RPS, Wash buffer (WB), Wash buffer E (WBE), Carbon beads, BB, NB, and elution buffer (EB).

Procedure

1) Once you get the aqueous phase using Trizol/phenol/chloroform method, proceed to step 2. Reagent volumes are based off of the amount of aqueous phase obtained. The volumes in this protocol assume 150 µL of aqueous phase, for other volumes adjust according to the quick reference table at the end of this document.
2) Add 30 µL RPS to the aqueous phase, mix and incubate for 5 mins at RT.
3) Add 70 µL carbon-beads to the above solution, mix and incubate for 2 mins, shake carbon beads immediately before using mix well.
4) Add 250 µL of binding buffer to the above solution. Mix well by pipetting up and down at least 8 times, pipette up and down at least 8 times in every step below that requires "mixing well." At this point you should start seeing beads clumping together as shown in Figure 1, this is the RNA binding. The clumps may break up as mixing continues.

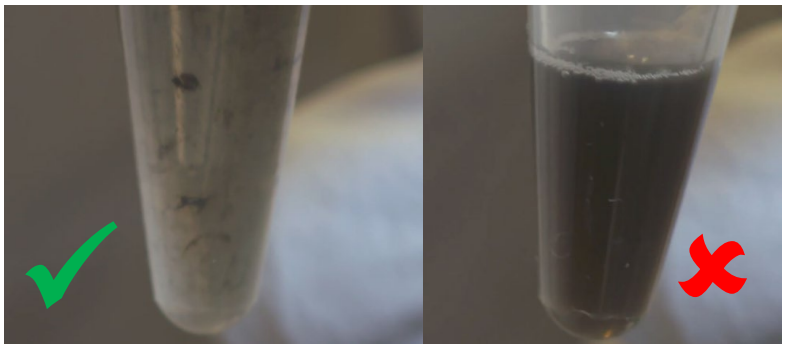

Figure 1: Picture of floccule formation after step 4 using cultured cells as the source sample.

5) Add 100 µl of diluted neutralization buffer solution and mix.
6) Place on magnetic stand for 90 secs and then remove supernatant.
7) 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.
8) Wash the beads two more times with wash buffer (WB). Add 750 µL of wash buffer and mix 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. 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.
9) Dry the bead for 10 mins (will dry faster if placed in a hood)
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 centrifugation step, place tube on magnetic stand and collect the clear liquid which contains pure RNA.

Reagent Volume Quick Reference

	Tissue	H_2O Phase	RPS	Carbon Beads	BB	NB	RPSWB	WB	Elution
Example*	5 mg	150 µL	30 µL	70 µL	250 µL	100 µL	750 µL	750/500 µL	30-100 µL
Ratio**	< 1 mg	1	0.2	0.46	1.66	50 µL	750 µL	750/500 µL	30-100 µL
	1-10 mg	1	0.2	0.46	1.66	100 µL	750 µL	750/500 µL	30-100 µL
	10-20 mg	1	0.2	0.46	1.66	150 µL	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, Carbon beads, and RBS 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. NB is scaled to the amount of input tissue. If you are using millions of cells as input, <1 Million cells, 1M to 7M, and >7M cells can be used as the three input tiers.**