Quick-Start Protocol

CarbonPrep Blood/OmniLyse Homogenizer

Shelf-life

- Carbon beads have a shelf-life of >2 years.
- RNA pre-binding solution (RPS), Wash Buffer (WB), Wash Buffer E (WBE) and Binding buffer (BB) have a shelf life of 1 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, Carbon beads, RPS, RPS Wash buffer, Wash buffer, BB, NB, and elution buffer (EB). You will also need Proteinase K and an OmniLyse Homogenizer, which are not included in this kit.

Procedure

1) This protocol assumes 150 µL of blood as the input, for other sample volumes see the table at the end of the document. Take 70 µL of carbon beads and place them in an empty vial and place the vial on a magnetic stand. Remove the clear liquid after the beads have separated.
2) Add 15 µL of Proteinase K (PK) to the blood sample and lyse the sample with the OmniLyse Homogenizer.
3) Add 70 µL of RPS to the sample and mix, incubate for 5 minutes.
4) Transfer the sample to the tube containing the beads which was prepared in step 1, remove the tube from the magnetic stand and mix well. 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." This makes an even dark solution. Incubate for 2 min.
5) Add 250 µL of binding buffer (BB) to the above solution, mix well. At this point you should start seeing beads clumping together as shown in Figure 1, this is the RNA binding. The clumps may disperse with further mixing.

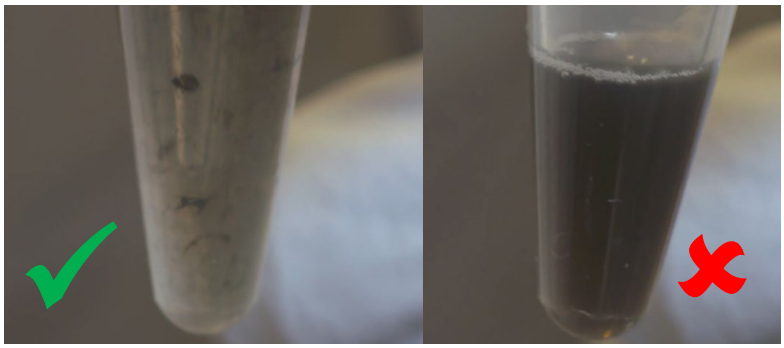

Figure 1: Picture of floccule formation after step 5 using cultured cells as the source sample.

6) Add 70 µl of diluted neutralization buffer solution and mix well.
7) Place on magnetic stand for 90 secs and then aspirate out the supernatant.
8) 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.
9) 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. Then wash the beads one last time 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

	Blood	PK	RPS	Carbon Beads	RBS	NB	RPSWB	WB	Elution
Example*	150 µL	15 µL	70 µL	70 µL	250 µL	70 µL	750 µL	750/500 µL	30-100 µL
Ratio**	1	0.1	0.46	0.46	1.66	0.46	750 µL	750/500 µL	30-100 µL

***The above protocol uses 150 µL of blood as an example.**
****For other amounts of blood, use the table. Proteinase K (PK), RPS, Carbon beads, RBS, and NB volumes should be scaled to the amount of aqueous phase obtained. For example, if 200 µL of blood is the input, use 92 µL of RPS.**