Quick-Start Protocol

CarbonPrep Animal Tissues

Shelf-life

- Carbon beads have a shelf-life of at least two years.
- RNA lysis buffer (Lysis Buffer), RNA pre-binding solution (RPS), RPS wash buffer (RPSWB), wash buffer(WB), elution buffer, 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.
- Salts may precipitate out of the lysis buffer, particularly at cold temperatures, warm the lysis buffer if it's cold.

Procedure

1) Reagent volumes are based off of the amount of tissue. This protocol assumes 8 mg of tissue. 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). You may also optionally add 15 µl of Proteinase K (PK) when working with fibrous tissues.
2) Add 150 ul of Lysis Buffer to sample.
3) Homogenize the tissue thoroughly. Make sure no visible clumps are present and the buffer is no longer viscous. (Note: If the lysate is too viscous, incubate at room temperature for few minutes and homogenize again).
    a. If using a TissueLyser:
        i. Add two 5mm stainless steel beads to your tube
        ii. Oscillate for 1 minute
            1. if warm, cool on ice for 1 minute
        iii. repeat a total of 3 times
        iv. Spin down samples using centrifugation for 2 minutes at 10,000g
        v. Separate out solution from beads as working solution
4) Add 5 ul of RPS to the above lysate, mix and incubate for 2-3 mins at RT.
5) To a fresh tube, add 70uL of LM beads and place on magnet
    a. Allow separation and remove supernatant
6) Add solution to the dry beads and mix thoroughly

a. allow to sit at room temperature for 5-10 minutes

7) Add 155 ul 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 properly)

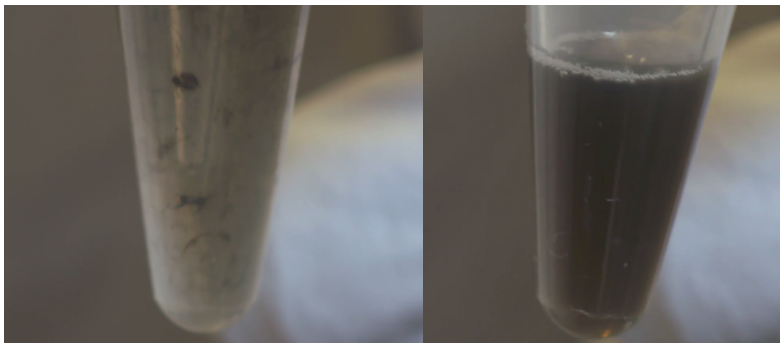

Figure 1: Picture of floccule formation after step 6 using cultured cells as the source sample. Clumps may break up after initially forming.

8) Place the tubes on the magnetic stand for 90 seconds and aspirate out the supernatant.
9) 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.
10) Wash the beads two more times with wash buffer (WB).
    a. 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.
    b. 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.
11) 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.
12) 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.
13) After heating, mix/flick samples. Then centrifuge at 10,000 g for 2 min to separate beads from RNA elute.
14) After centrifugation step, place tube on magnetic stand and collect the clear liquid which contains pure RNA.

Reagent Volume Quick Reference

	mg of tissue	Lysis Buffer	RPS	Carbon Beads	BB	WB	Elution
Example*	8 mg	150 µL	30 µL	70 µL	300 µL	750/500 µL	30-100 µL
Ratio**	<1 mg	125 µL	25 µL	60 µL	250 µL	750/500 µL	30-100 µL
	1-8 mg	150 µL	30 µL	70 µL	300 µL	750/500 µL	30-100 µL
	>8 mg	200 µL	40 µL	90 µL	400 µL	750/500 µL	30-100 µL

***The above protocol uses 8 mg of tissue as an example. For other amounts, use the values in the table.**

****For tissues less than 1 mg it may be desirable to scale the reaction volumes proportionally**