

## Quick-Start Protocol

# CarbonPrep Cultured Cells - Rebinding

### Shelf-life

- Carbon beads have a shelf-life of at least two years.
- RPS (pre-binding agent), lysis buffer, 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.

### Procedure

***Reagent volumes are based on the concentration of RNA. This protocol assumes 10-15 µg total isolated RNA.***

- Add 1 µL of RPS to 50 µL of isolated RNA.
  - a. Incubate for 10 minutes at RT
- Add 50 µL of 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 equal parts of the binding buffer directly to the RNA-bead solution and mix well by pipetting up and down. (With pure RNA solution as your starting material, the sample will appear dark.)
- 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  $\mu\text{L}$  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  $\mu\text{L}$  of elution buffer, 50  $\mu\text{L}$  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.

### FAQs

1. Rebinding is best done when the lysis buffer used in original isolation is GITC.
2. If you experience a low yield, increase the volume of beads to 75 $\mu\text{L}$  or 80 $\mu\text{L}$ .
3. RNA rebinding with LM kits will clean up all DNA contamination present in the sample. See example below.

