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 uL 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 µL 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 µL of elution buffer, 50 µL 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 75µL or 80µL.
3. RNA rebinding with LM kits will clean up all DNA contamination present in the sample. See example below.