Quick-Start Protocol

CarbonPrep Cultured Cells

Upon receiving your kit

- Add molecular biology grade ethanol to each bottle which requires it. Check the boxes when this is complete.
- Neutralization buffer should be stored at 4 °C long term but is stable at RT for at least 3 months.

Preparing for extractions

- Dilute neutralization buffer 1:10 with molecular biology grade water. You will use 100 µL per reaction in step 5. The elution buffer is molecular biology grade water and can be used for dilution.
- In all mix steps, do at least 6 pipette strokes for best results.
- Carefully remove all cell wash buffer from pellet before starting. Cell wash buffer is PBS and PBS reduces RNA yield with every RNA isolation kit we've tested! Remove as much PBS as possible.
- **Optional:** To maximize yield, add 10 µL of proteinase K (20 mg/ml) per 150 µL of lysis buffer. You will use 150 µL of lysis buffer per reaction. Proteinase K is not provided with this kit.
- **Optional:** If purifying RNA from cell lines rich in RNase, add 2 µL of β-mercaptoethanol (β-ME) per reaction. β-ME is not provided with this kit.

Procedure

1) **Lysis:** add 150 µL of lysis buffer to a cell pellet containing 2 to 5 million cells. Refer to table 1 if the sample input falls outside of this range.
2) Lyse the sample by syringe, pipetting up and down, or vortexing. Homogenize the sample until it is no longer viscous. Incubate for 3 min at room temperature. Other homogenization methods such as bead beating, sonication, spinning, ect. are also acceptable as long as the lysate is homogenous and no longer viscous. If DNA contamination is observed or RNA yield is low, consider changing methods.
3) Add 70 µL of carbon magnetic beads and mix well, creating an even dark solution.
4) **Binding:** Add 230 µL of binding buffer and mix well immediately. The texture of the carbon beads will change. This is RNA binding to the beads.
5) Add 100 µL of neutralization buffer mix well. This helps release any trapped DNA.

6) **Washing:** Add 750 μL of RPS Wash Buffer (WBRPS) and mix well. Place on a magnetic stand for 45 secs or until black aggregates are collected, and then remove supernatant.
7) Add 750 μL of Wash Buffer (WB) and mix well. Place on a magnetic stand for 45 secs or until black aggregates are collected, and then remove supernatant.
8) Add 500 μL of Wash Buffer (WB) and mix well. Place on a magnetic stand for 45 secs or until black aggregates are collected, and then remove supernatant. For best results, transfer the mixture to a fresh 1.5 ml tube.
9) Remove all liquid and then dry the beads for 10 mins (will dry faster if placed in a hood). Remove liquid again after a few minutes; residual liquid on walls may pool at the bottom of the vial. Ethanol promotes binding so dry beads long enough such that the ethanol evaporates but not so long such that the water also evaporates.
10) After drying add 30 - 100 μL of elution buffer, 50 μL is recommended. Heat the sample at 65 °C for 5 min to fully elute RNA.
11) After heating, mix/flick samples. Then centrifuge at 10,000 g for 2 min to separate beads from RNA elute. Alternatively, place the tube on a magnetic stand and wait 10 minutes for the beads to fully separate.
12) Place tube on magnetic stand and collect the clear liquid which contains pure RNA.

Reagent Volume Quick Reference

Input Millions of Cells	Lysis Buffer	Carbon Beads	BB	NB
3 M	150 µL	70 µL	230 µL	100 µL
<1 M	100 µL	40 µL	150 µL	50 µL
1 to 5 M	150 µL	70 µL	230 µL	100 µL
>5 M	200 µL	70 µL	280 µL	100 µL

***Reagents not listed, including neutralization buffer, wash buffers, and elution buffers do not change volume based on input material.**

****If you are using <100,000 cells, see our low volume protocol**