Quick-Start Protocol

CarbonPrep Animal Tissue

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

- In all mix steps, do at least 6 pipette strokes for best results.
- **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 tissues 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 2 m to 8 mg of tissue. 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 60 µL of carbon magnetic beads and mix well, creating an even dark solution.
4) Add 40 µL of RPS A buffer, mix and incubate for 2-3 min at RT.
5) **Binding:** Add 250 µL of binding buffer and mix well immediately. The texture of the carbon beads will change. This is RNA binding to the beads.
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). After a few minutes, remove liquid again; 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

mg of tissue	Lysis Buffer	RPS A	Carbon Beads	Binding Buffer
<2 mg	125 µL	30 µL	50 µL	200 µL
2-8 mg	150 µL	40 µL	60 µL	250 µL
>8 mg	200 µL	60 µL	70 µL	350 µL

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