Quick-Start Protocol

CarbonPrep Trizol/Phenol

Upon receiving your kit

- Add molecular biology grade ethanol to each bottle which requires it. Check the boxes when this is complete.

Preparing for extractions

- Take 70 μL of carbon beads, place them in a magnetic stand. After the beads have separated, remove the clear storage buffer. You will use the beads in step 2.
- Dilute neutralization buffer 1:10 with molecular biology grade water. You will use 100 μL per reaction in step 6. 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.
- If using cultured cells, 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.

Procedure

For remote collection, you may combine steps 1 and 2 by adding the lysis buffer and carbon beads together and then adding the urine to that mixture.

1) **Lysis:** add 150 μL of urine to 150 μL of lysis buffer
2) Add the above mixture to the tube containing the separated magnetic beads
3) Incubate at RT for 30 min with occasional mixing. If you are shipping the sample, this will occur during normal shipping conditions. RNA is stable for 7 days at RT.

If the sample is collected remotely the following steps are performed when the sample reaches the lab for analysis.

4) **Add ethanol:** Add 300 μL of binding buffer and mix well immediately. The texture of the carbon beads will change. Mix immediately after adding ethanol to avoid creating pockets of high ethanol concentration that denature DNA and lead to DNA binding.
5) Place the tubes on a magnetic stand and aspirate out the supernatant.

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

Urine	Lysis Volume	Carbon Beads	Ethanol
150 µL	150 µL	70 µL	300 µL
300 µL	300 µL	140 µL	600 µL

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