Quick-Start Protocol

CarbonPrep Viral

Upon receiving your kit

- Add molecular biology grade ethanol to each bottle which requires it. Check the boxes when this is complete.
- We recommend 96-well plate magnetic stands in a 24 post style or 6 bar style.
- Combine Trizol with RPS Viral. Add 7 μL of RPS viral for every 100 μL of Trizol.

Setup before performing 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 this in step 2.
- In all mix steps, do at least 6 pipette strokes for best results.
- You will need 200 proof molecular biology grade ethanol in step 3.

Procedure

1) **Lysis:** add 107 μL of Trizol with RPS V to 100 μL of sample, such as saliva or viral transport media (VTM). For other volumes of input material, refer to the table below. Incubate for 5 minutes. Mix every 90 second.
2) Transfer the solution to the tube containing the carbon beads. Mix well by pipetting up and down. Incubate for 4 min at RT.
3) **Binding:** Add 200 μL of ethanol and mix well immediately. The texture of the carbon beads will change. This is RNA binding to the beads.
4) **Washing:** Add 480 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.
5) Add 480 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.
6) Add 160 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 and to ensure all protein/DNA contamination is removed, you can transfer the mixture to a fresh 1.5 ml tube.
7) 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.

8) After drying add 30 - 100 μL of elution buffer, 30 μL is recommended. If performing manual extractions, mix and heat samples for 5 min at 65 degree to promote RNA release. On automated instruments, release can be achieved by vigorous mixing.
9) After heating, mix/flick samples. If performing manual extractions, then centrifuge at 10,000 g for 2 min to separate beads from RNA elute. On automated instruments, a magnet can be used but use a long wait time (>5 min) to ensure beads are fully separated.
10) After centrifugation step, place tube on magnetic stand and collect the clear liquid which contains pure RNA.

Reagent Volume Quick Reference

Saliva or VTM*	Trizol Volume	RPS V	Ethanol
100 µL	100 µL	7 µL	200 µL
150 µL	150 µL	11 µL	300 µL
200 µL	200 µL	14 µL	400 µL

***Viral transport media**