

**RNA EXTRACTION USING PetNAD Nucleic Acid Co-prep Kit
(GeneReach Biotechnology Corp. Cat. # apet-ext)**

Wear gloves throughout this procedure to avoid contaminating the samples with RNase which will degrade the RNA being extracted.

item	Volume/bottle	Note: before first use
PB1	36 ml	N/A
PB2	1 ml	Add 35 ml 95% ethanol before starting
PB3	20 ml	Add 20 ml 95% ethanol before starting
PB4	15 ml	Add 25 ml 95% ethanol before starting
PB5	12 ml	N/A

Procedure:

1. Grind ~40 mg of tissues in 500 µl PBS buffer
2. Remove 200 µl of supernatant and mix with 600 µl of **PB1** buffer for 1 min.
3. Add 600 µl **PB2** buffer (with ethanol), mix thoroughly for 10 sec.
4. Transfer 600 µl of mixture to a spin column & collection tube
5. Spin at full speed for 1 min, discard the flow-through and recombine the column & collection tube.
6. Add 600 µl **PB3** buffer (with ethanol) to the column, spin for 1 min, discard flow-through, recombine the column & collection tube.
7. Add 600 µl **PB4** buffer (with ethanol) to the column, spin for 1 min, discard the flow-through, recombine the column & collection tube.
8. Spin for additional 3 min to dry the column.
9. Transfer the column to a clean 1.5 ml Eppendorf tube, add 50-200 µl **PB5**, incubate at room temperature for 1 min.
10. Spin for 1 min to elute the nucleic acid (both **RNA** and DNA).

A. Quick Guide

