

RNA EXTRACTION WITH THE QIAzol RNEASY LIPID TISSUE MINI KIT (Cat. No. / ID: 74804)

Add 4 volumes of ethanol (96-100%) to buffer RPE before you use RNeasy Lipid Tissue Mini Kit.

1. Add 1 ml QIAzol Lysis Reagent per 100 mg tissue to an appropriate vessel for homogenization and subsequent centrifugation. The volume of tissue should not exceed 10% of the volume of QIAzol Lysis Reagent. For micropunches and small samples, add 40 microliters of QIAzol Lysis reagent.
2. Incubate the homogenate at room temperature (15-25°C) for 5min.
3. Add 1/5 of volume of chloroform (e.g. for 40 microliters of QIAzol Lysis add 8 microliters of chloroform) and shake vigorously for 15s.
4. Incubate sample at room temperature for 2-3min.
5. Centrifuge at 12000 x g (~13500 rpm in the Eppendorf 5424R) for 15min at 4°C. Let the centrifuge heat to room temperature.
6. Transfer upper, aqueous phase to a new tube. Be careful to avoid the interphase.
7. Add approximately the same volume of the aqueous phase of 70% ethanol (usually 25 microliters for an initial volume of 40 microliters) and vortex. Do not centrifuge.
8. Transfer to RNeasy mini spin column and centrifuge for 15sec at 8000 x g (~10000 rpm) at room temperature, subsequent discard the flow-through.
9. Repeat step 9 using the remainder of the sample. Discard the flow-through.
Optional DNase digest: Follow steps in “Optional on-column DNase digestion with the RNase-free DNase Set” in Appendix C of the RNeasy Lipid Tissue Kit Handbook.
10. (skip this step if performing optional DNase digestion). Add 700 microliters Buffer RW1 to RNeasy column, close lid and centrifuge for 15sec at 8000 x g (~10000 rpm), discard the flow-through.

11. Add 500 microliters Buffer RPE to RNeasy column, close lid and centrifuge for 15sec at 8000 x g (~10000 rpm), discard the flow-through.
12. Add 500 microliters Buffer RPE to RNeasy column, close lid and centrifuge for 2min at 8000 x g (~10000 rpm), discard the flow-through.
13. To further dry membrane, place RNeasy column in new 2ml tube, close lid and centrifuge for 1min at 13500 rpm, discard the flow-through.
14. Place RNeasy column in a new 1.5ml tube, add 30-50 microliters RNase – free water, close lid and centrifuge for 1min at 8000 x g (~10000 rpm). Store the flow-through at -80°C.