



T001.0P - Qiagen RNeasy Mini for Isopropanol Samples Protocol

(4-10-10, Lynn Law)

This protocol is used when RNA samples are received from collaborators in the form of isopropanol precipitations in microfuge tubes.

1. Thaw tubes and centrifuge at 12,000g for 15min 4⁰C
2. Remove and save the Isopropanol
3. Add 350µl RLT + β-ME to the pellet
4. Mix well and make sure the pellet is dissolved before adding 70% ethanol.
5. Add 350µl 70% ethanol and mix well- Do not spin at this point.
6. Transfer the 700µl of sample to an RNeasy spin column and centrifuge for 15sec at 8,000g (10,500 rpm). Discard the flow-through
7. Added 700µl of RW1 buffer to column and centrifuge for 15sec at 8,000g (10,500 rpm) Discard the flow-through
8. Add 500µl of RPE buffer to column and centrifuge for 15sec at 8,000g (10,500 rpm) Discard the flow-through
9. Add 500µl of RPE buffer to column and centrifuge for 2min at 8,000g (10,500 rpm) Discard the flow-through
10. Centrifuge at 8,000g (10,500 rpm) for 1min * Perform this step to eliminate any possible carryover of Buffer RPE, or if residual flow-through remains on the outside of the RNeasy spin column
11. Place the RNeasy spin column in a 1.5ml collection tube and add 30µl RNase-free water directly to the spin column membrane then centrifuge for 1min at 8,000 x g (10,500 rpm) to elute the RNA * If the expected RNA yield is >30µg, repeat this step using another 30µl RNase-free water. Store RNA solution at - 80C.

