**Alternative step by step protocol: chromotography colu based total RNA isolation for single worm quantitative RT-PCR**

It's convenient, in comparison with Trizol based method, although it's somehow limited in detection of low abundance transcript even the abuandant internal control works well in our hands (not shown).

1. Washed by M9 and 70% ethanol, pick single worm into 350µl RLT (QIAGEN), frozen at -70°C, then thawn for 3 times.
2. Centrifuge the lysate for 3 min at 14000rpm at 4°C. Carefully transfer the supernatant to a new eppendorf tube.
3. Add 350µl of 70% ethanol to the lysate, and mix immediately by pipetting.
4. Apply up to 700µl of sample to an RNeasy mini column placed in a 2 ml collection tube. Close the tube tightly, then centrifuge for 15s at 14000rpm. Discard the flowthrough. Re-use the collection tube in step 5.
5. Add 700µl RW1 (QIAGEN), to the RNeasy column (QIAGEN). Close the tube gently, and centrifuge for 15s at 14000rpm. Discard the flowthrough and collection tube.
6. Transfer the RNeasy column into a new 2 ml collection tube. Pipet 500µl RPE (QIAGEN) onto the RNeasy column. Close the tube and centrifuge for 15s at 14000rpm. Discard the flowthrough and re-use the collection tube.
7. Add another 500 µl RPE to the RNeasy column. Close the tube gently, and centrifuge for 3min at 14000 rpm .
8. To elute, transfer the RNeasy column to a new 1.7 ml collection tube. Pipet 30-50µl RNease-free water directly onto the RNeasy membrane. Close the tube gently, and centrifuge for 1 min at 14000rpm to elute.