

## **Protocol: MTB Total RNAs Isolation (Alternative Qiagen Purification Method)**

Stanford protocol adapted from Qiagen miRNA and RNAeasy manuals – updated 9.2.09

This protocol is used for the isolation of small RNAs by elimination of the isopropanol precipitation step and modification of the Qiagen RNAeasy mini kit.

### **1. Total RNAs Purification:**

1. Place aqueous phase tubes on ice upon removal from the BL3.
2. Add **1.5 volumes** of **100% EtOH** to the aqueous phase. Mix well.  
*i.e. 600  $\mu$ l aqueous phase + 900  $\mu$ l 100% ethanol*
3. Prepare the Qiagen RNAeasy columns and label appropriately.
4. Pipette **700  $\mu$ l** of the aqueous phase mix directly into the corresponding column.
5. Centrifuge for **30 sec**. A vacuum manifold can be used if multiple samples are processed at once. Repeat steps 4 and 5 until the whole sample has been pipetted into the spin column. Discard the flow-through each time.
6. Place columns in a new waste collection tube.
7. Pipette **700  $\mu$ l** of **RWT** buffer directly into each column.  
Note: **RWT** buffer is provided with the Qiagen miRNA kit.
8. Centrifuge for **30 sec**.
9. Place columns in a new waste collection tube. Discard the flow-through.
10. Pipette **500  $\mu$ l** of **RPE** buffer directly into each column.
11. Centrifuge for **30 sec**.
12. Discard the flow-through and reuse the collection tube or transfer column to a new waste collection tube.
13. Repeat wash with **500  $\mu$ l** of **RPE** buffer. Centrifuge for **2 min**.
14. Place column in a new waste collection tube.
15. Centrifuge for **1 min**.
16. Place column in a new 1.5 ml elution tube. Open cap and let column air-dry for **2 min**.
17. Elute in specified volume (depending on expected concentration and whether this is the 1<sup>st</sup>/2<sup>nd</sup> DNase treatments or post-DNase treatments). Typically, elute RNAs to **84  $\mu$ l** before DNase treatment.  
*i.e. if eluting 84  $\mu$ l total, elute in two steps with 50  $\mu$ l first followed by a 34  $\mu$ l second elution.*  
RNAs are eluted in either the provided RNase-free water or TE pH 7.0 buffer (Ambion, Cat. AM9860).

### **2. OFF-column RQ1 DNase:**

For downstream RT-PCR, 2x-DNase treatments are often necessary to eliminate DNA contamination. DNA contamination can be tested by running assays with and without reverse transcriptase during the cDNA synthesis procedure.

1. OFF-column RQ1 DNase: to **84  $\mu$ l** of newly prepared RNA, add **10  $\mu$ l** of 10x RQ1 DNase I buffer, **2.5  $\mu$ l** of Ribolock Inhibitor (Fermentas, EO0382, 40 U/ $\mu$ l) and **4  $\mu$ l** of RQ1 DNase (*Promega, M6101, 1000U*). *DNase is unstable to shear forces & the stock of it should be treated carefully and replaced upon expiration.*
2. Flick gently with finger to mix. If spin is needed, quick spin no more than **3 sec**. Incubate for **30 min** in 37°C bath.

3. **Column clean up:** to 100  $\mu\text{l}$  of sample (from step 18), add 350  $\mu\text{l}$  of fresh RLT buffer (+ 10  $\mu\text{l}/\text{ml}$   $\beta$ -Mercapto ethanol per ml of RLT buffer) and pipette thoroughly to mix. Add 700  $\mu\text{l}$  of 100% EtOH and pipette thoroughly to mix.
4. Pipette 700  $\mu\text{l}$  of the mix to a new RNeasy column. Centrifuge for 30 sec.
5. Transfer RNeasy column to a new 2 ml collection tube. Wash by adding 500  $\mu\text{l}$  of buffer **RPE** to the column. Centrifuge for 30 sec. Discard the flow-through.
6. Repeat wash with 500  $\mu\text{l}$  of **RPE**. Centrifuge for 2 min.
7. Transfer column to a new collection tube and centrifuge for 1 min to dry column membrane.
8. Transfer column to an Rnase-free 1.5ml Eppendorf tube (from kit), open cap of column and allow membrane to air dry for 2 min.
9. Elute sample in either 84  $\mu\text{l}$  (if performing a 2<sup>nd</sup> DNase treatment) or 50  $\mu\text{l}$  (if after 2<sup>nd</sup> DNase treatment).
10. For 2<sup>nd</sup> DNase, repeat steps 18-26.
11. Store 2x-DNAsed RNA samples in -80°C freezer.