

## Protocol: Host RNA Isolation from MTB-infected Lung Tissue and Sputa

Host RNA can be extracted along with MTB RNA during the RNA isolation process. This protocol can be performed at the same time as MTB RNA isolation or the supernatant containing host RNA can be stored at -80°C for later use.

### 1. Isolation of Eukaryotic host RNA : lung tissue or sputa

Instead of discarding the supernatant during the RNA isolation process, supernatant containing host RNA can be isolated further.

*Note: Since host RNA is present in excess quantity, process only 1ml from total, store remaining volume at -80°.*

- Transfer 1 ml of supernatant to a 2 ml screw cap tube.
- Centrifuge 1 min. at maximum speed (*Eppendorf 5415D*).
- Transfer supernatant to a new 2 ml screw cap tube containing Heavy Phase Lock Gel.
- Add 300 µl chloroform.
- Vortex tube for 15 sec. Continue to vigorously invert tube for 2 min.
- Centrifuge for 10 min. at maximum speed (*Eppendorf 5415D*).
- Transfer the aqueous phase (~540 µl) to a 2.0 ml elution tube (*Ambion Cat#12480*).
- Place on ice.

### 2. Decontaminate tube(s) by wiping with Staphene and remove from BL3.

### 3. Total Eukaryotic RNA:

This step is similar to the precipitation and purification of MTB RNA.

#### A. Precipitation of aqueous phase:

- Add Glycoblue coprecipitant (*Ambion Cat#9515, 15mg/ml*) at 150µg/ml final concentration. Glycoblue consists of a blue dye covalently linked to glycogen; it improves visibility of RNA pellet upon precipitation.  
*i.e. 500 µl aqueous phase + 10 µl Glycoblue.*
- Add 1:10 volume of 5M ammonium acetate. Mix well.
- Add 1:1 volume of isopropanol to aqueous phase. Mix samples repeatedly by inversion. *i.e. 500 µl aqueous phase + 500 µl isopropanol*
- Incubate at room temperature for 20min. Alternatively, samples may be placed at -20c overnight, if not processed immediately.
- Centrifuge at 4°C for 10min. Blue RNA pellet should be visible on the side of tube.
- Decant isopropanol. Wash twice in 70% ethanol. Spin for 5min. after each wash.

#### B. RNeasy column followed by OFF-column RQ1 DNase (1x DNase):

- Remove trace ethanol. Reconstitute by adding 100 µl **Rnase-free water**.
- **Column clean up:** to 100 µl of sample, add 350 µl of *fresh* RLT buffer (+ 10 µl/ml β-Mercapto-ethanol + 20 µg/ml linear acrylamide) and pipette thoroughly to mix. Add 250 µl of **100% EtOH** and pipette thoroughly to mix.

- Add to a new RNeasy column. Centrifuge for 30 sec.
- Transfer RNeasy column to a new collection tube. Wash by adding 700  $\mu$ l of buffer **RW1** to RNeasy column and centrifuge for 30 sec. Discard eluate. Repeat wash with 700  $\mu$ l of buffer **RW1** and centrifuge a second time for 30 sec.
- Transfer RNeasy column to a new 2 ml collection tube. Wash by adding 500  $\mu$ l of buffer **RPE** (ensure that EtOH has been added to this buffer) to RNeasy column and centrifuge for 30 sec. Discard eluate. Repeat wash with 500ul of **RPE** and centrifuge a second time for 2 min.
- Transfer column to a new collection tube and centrifuge for 1 min to dry column membrane.
- 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.
- Elute sample by adding 50  $\mu$ l of **RNase-free water**. Do not close cap, let membrane soak for 2 min. Close cap and centrifuge for 2min. Perform a second elution in the same collection tube with 34  $\mu$ l of **RNase-free water** and centrifuge for 2 min. Total sample volume should be 80  $\mu$ l.
- **OFF-column RQ1 DNase:** add 10  $\mu$ l RQ1 buffer, 2.5  $\mu$ l Ribolock RNase inhibitor (Fermentas, Cat. No. EO0381), and 8  $\mu$ l RQ1 DNase (Promega, Cat. No. M6101). Mix by gently flicking side of tube until mixture is homogenous.
- Incubate in 37c water bath for 30 minutes.
- **Column clean up:** to 100  $\mu$ l of sample (from step 14), add 350  $\mu$ l of *fresh RLT* buffer (+ 10  $\mu$ l/ml  $\beta$ -Mercapto ethanol + 20  $\mu$ g/ml linear acrylamide) and pipette thoroughly to mix. Add 250  $\mu$ l of **100% EtOH** and pipette thoroughly to mix. Add to a new RNeasy column. Centrifuge for 30 sec.
- Transfer RNeasy column to a new 2 ml collection tube. Wash by adding 500  $\mu$ l of buffer **RPE** (ensure that EtOH has been added to this buffer) to RNeasy column and centrifuge for 30 sec. Discard eluate. Repeat wash with 500ul of RPE and centrifuge a second time for 2 min.
- Transfer column to a new collection tube and centrifuge for 1 min to dry column membrane.
- 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.
- Elute sample by adding 50  $\mu$ l of **RNase-free water**. Do not close cap, let membrane soak for 2 min. Close cap and centrifuge for 2min. Perform a second elution in the same collection tube with 34  $\mu$ l of **RNase-free water** and centrifuge for 2 min. Total sample volume should be 80  $\mu$ l.
- **2<sup>nd</sup> OFF-column RQ1 DNase:** Repeat OFF-column RQ1 DNase treatment (total 2x DNase). Refer to procedures above.
- Elute sample by adding 30  $\mu$ l of **RNase-free water**. Do not close cap, let membrane soak for 2 min. Close cap and centrifuge for 2min. Perform a second elution in the same collection tube with 20  $\mu$ l of **RNase-free water** and centrifuge for 2 min. Total sample volume should be 50  $\mu$ l.
- Store in -80°C freezer.