

# **Malaria Total RNA Isolation**

(04/26/02)

## **Harvesting the sample:**

- 1) Centrifuge the sample at 1500 rpm for 5 minutes with no brake in table top centrifuge.
- 2) Aspirate off the supernatant and wash the cells with 1X PBS (25 ml per 1ml pellet).
- 3) Centrifuge the sample at 1500 rpm for 5 minutes with low brake in table top centrifuge.
- 4) Aspirate off the supernatant and drop the tube into liquid nitrogen right away. Store at  $-80^{\circ}\text{C}$  until ready to isolate RNA.

## **RNA Isolation:**

- 1) Thaw out the cells in  $65^{\circ}\text{C}$  water bath for 1 minute.
- 2) Add 10 ml TRIZOL (stored at  $4^{\circ}\text{C}$ ) (Invitrogen 10296-028) and pipette up and down until the cells are resuspended well. (Usually about 10 times ok.)
- 3) Add 2 ml chloroform (Fisher BP1145-1) and shake well. Let sit for 5 minutes on ice.
- 4) Centrifuge at 3000 rpm for 10 minutes NO (or low) brake in table top centrifuge.
- 5) Transfer the supernatant to a 14 ml FALCON Polypropylene round-bottom tube and measure the volume.
- 6) Add  $1/10^{\text{th}}$  this volume of 3M NaOAc, pH 5.5 and add equal volume of isopropanol (stored at  $-20^{\circ}\text{C}$ ). Shake well to mix. Precipitate overnight at  $-20^{\circ}\text{C}$ .
- 7) Centrifuge at 9000 rpm in SS34 rotor (with adaptors) for 1 hour at  $4^{\circ}\text{C}$ .
- 8) CAREFULLY pour out the supernatant. (You should see a pellet at this stage.)
- 9) Wash the pellet with 10 ml 70% Ethanol and let sit on ice for 10 minutes.
- 10) Centrifuge at 9000 rpm in SS34 rotor (with adaptors) for 10 minutes at  $4^{\circ}\text{C}$ .
- 11) Again, carefully pour out the supernatant.
- 12) Dry the pellet in the speed vac for 3 minutes.
- 13) Resuspend the pellet in 100 ul GOOD water.
- 14) Store RNA at  $-80^{\circ}\text{C}$ .

## **RNA quantitation:**

- 1) Measure OD 260 and ratio of 260/280. (2 ul in 120 ul water)
- 2) Run 1 ul of RNA on a 1% agarose gel.