

RNA Extraction

Reagents:

- Parasite Cultures (~200mL of 8-12% parasitemia cultures)
- Trizol LS; [Gibco]
- Isopropanol (RNase-free)
- RNeasy Kit; [Qiagen]
- Agarose/Formaldehyde Gel

For a 1.5% agarose formaldehyde gel...

1. Add 0.6g agarose in 29mL ddH₂O. Dissolve in microwave, cool slightly.
2. Add 4mL 10x electrophoresis buffer (0.2 M MOPS pH 7.4 and 10mM EDTA)
3. Add 7mL formaldehyde under hood.
4. Add EtBr.
5. Mix quickly and pour.

Protocol:

1. Resuspend cells and transfer to a 50mL sterile falcon tube.
2. Centrifuge 800xg 5' @ RT.
3. Remove supernatant and transfer 0.5 -1mL of infected blood into a sterile RNase-free Nalgen tube.
4. Add 10-20 volumes (10 for rings, 15 for trophozoites, 20 for schizonts) of pre-warmed (37°C) Trizol LS.
5. Mix samples thoroughly to dissolve all clumps.
6. Leave @ 37°C 5', then store @ -80°C immediately if needed.
7. For each 5mL of Trizol LS add 1mL chloroform and mix sample vigorously by shaking.
8. Centrifuge 12,000xg 30' @ 4°C.
9. Transfer the upper aqueous layer to a new RNase-free tube taking care to avoid the region just above the interface.
10. Mix the aqueous layer with 0.8 volumes isopropanol.
11. Split sample into 1.5mL RNase-free microcentrifuge tubes and leave on ice for at least 2hrs.
12. Centrifuge 2,000xg 30' @ 4.0°C.
13. Remove carefully the supernatant with a fine tip pipette.
14. Allow the RNA pellet to air dry for 5'.
15. Add 10-20µL RNase-free water to each tube.
16. Heat samples 10' @ 60°C, then place on ice.
17. Pool the split samples back into one RNase-free microcentrifuge tube.
18. Clean the total RNA using an RNeasy kit.
19. Read the OD₂₆₀/ OD₂₈₀. (Concentration should be 1-5µg/µL)
20. Check quality of RNA using an agarose formaldehyde gel.