| ACGT MICROARRAY FACILITY<br>**STANDARD OPERATING PROCEDURE** | | |
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| **TITLE: RNA isolation from *P.falciparum* infected erythrocytes** | | **PAGE: 1 of 2** |
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## 1. PURPOSE

This procedure describes RNA isolation from ***P.falciparum*** **infected erythrocytes** .

## 2. SCOPE

The starting material for this protocol is a pellet of *P.falciparum* infected erythrocytes (~0.5 ml), originating from 10 - 12 ml of *in vitro* culture (10% parasitaemia, 5% haematocrit), initially washed three times with 1x PBS and then frozen at -70 °C. The RNA isolation procedure is a combined RNeasy Mini Kit (QIAGEN) and TRI-Reagent (Sigma) method, which incorporates DNase 1 on-column digestion (QIAGEN). There is no saponin lysis of the erythrocytes.

## 3. MATERIALS

- RNeasy kit (QIAGEN; cat# 74104)
  TRI-Reagent (Sigma; cat# T9424)
- RNase free DNase1 set (QIAGEN; cat#79254)
  QIA-Shredder (QIAGEN; cat# 79654)

## 4. PROCEDURE

NOTE: ALL STEPS ARE AT ROOM TEMPERATURE

- 50 ml tubes containing frozen infected erythrocyte pellet is removed from -70 °C freezer and thawed.
- 600 µllysis buffer (RLT, from the RNeasy kit), is added and mixed by pipetting.
- The mixture is pipetted onto a QIA-Shredder column and centrifuged at 15700 g for 1 min.
- The flowthrough is transferred to clean microfuge tubes (2).
- 600 µlof TRI-Reagent is added to each tube and mixed by pipetting.
- The tubes are incubated at room temperature for 5 min.
- 400 µlof chloroform is added to each tube and mixed.
- The tubes are incubated at room temperature for 10 min.
- The tubes are then centrifuged at 15700 g for 15 min.

- The upper aqueous phase of each tube is transferred to clean microfuge tubes.
- 1 volume (700 µl)of 70% ethanol is added, mixed and the samples are loaded onto the RNeasy columns and centrifuged at 8000 g for 15 sec (only 700 µlcan be loaded at a time, so repeat this process until all the sample has been loaded. Use 1 column per sample).
- 350 µlof wash buffer RW1 was added and centrifuged at 8000 g for 15 sec.
- 70 µlof Buffer RDD is added to the 10 µlaliquot of DNase1. This 80 µlis the pipetted directly onto the membrane and incubated at room temperature for 15 min.



- 350 µlof wash buffer RW1 is added and centrifuged at 8000 g for 15 sec.
- 500 µlof wash buffer RPE is added and centrifuged at 8000 g for 15 sec.
- A further 500 µlof wash buffer RPE is added and centrifuged at 15700 g for 2 min.
- The column is transferred to a clean 1.5 ml microfuge tube.
- 30 µlof RNase free water is added to the column and centrifuged at 8000 g for 1 min to elute the RNA.
- The RNA was transferred to a clean microfuge tube and stored at -70 °C.