RNA Isolation Protocol

(Revised 5-15-2003)

Stabilize RNA

Start with 15 ml E. coli Culture containing 7.5* 10^9 cells (OD_{600}= 0.2 Dilute cells or scale up)

- Pipet 30 ml of RNAProtect Bacteria Reagent (Qiagen) into a 50ml polypropylene conical tube.
- Pipet 15 ml culture into the tube. Mix immediately by vortexing for 5second. Incubate for 5min at room temperature.
- Centrifuge for 10min at 5800g(4500rpm for H-6000A rotor in SORVALL RC-3B centrifuge)
- Decant the supernatant, and leave tubes inverted on a paper towel for 10s.
- Freeze the pellet with EtOH/Dye Ice mix.
- The pellet can be stored at -20°C up to 2 weeks, or -70°C for up to 4 weeks.

Isolation RNA

- Dilute 2ul of Proteinase K into 300ul of Tissue and Cell Lysis solution for each sample.
- Resuspend cell pellet by the Lysis solution and mix thoroughly. Transfer mix to 1.5ml tube.
- Incubate at 65°C for 45min, and vortex every 15min.
- Place the sample on ice for 5min.
- Add 150ul of MPC protein Precipitation Reagent to 300ul of lysed sample and vortex mix for 10sec.
- Spin for 10min 4°C at max speed in a microcentrifuge. Transfer the supernant to a clean tube.
- Add 50ul of MPC protein Precipitation Reagent and repeat above step.
- Add 500ul isopropanol to the recovered supernatant, invert the tube 30-40 times.
- Pellet the RNA by centrifugation at 4°C for 10min in a microcentrifuge.
- Carefully pour off the isopropanol without dislodging the RNA pellet. Remove all of the residual isopropanol with a pipet. Air dry 10-15min.

Removal of contaminating DNA

- Dilute 10ul of RNAse-Free DNAse I up to 200ul with 1x DNAse Buffer for each sample.
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Completely resuspend the nucleic acid pellet in 200ul of DNAse I solution.

- Incubation at 37deg;C for 45min
- Add 200ul of 2x T and C lysis solution, vortex mix for 5 seconds
- Add 200ul of MPC reagent vortex mix 10seconds, place on ice 5min.
- Pellet the debris by centrifugation for 10min at 4°C, >10,000g in a microcentrifuge.
- Add 50ul of MPC reagent and repeat 5 and 6 (but this time spin 20min).
- Add 600ul isopropanol to the recovered supernatant, invert the tube 30-40 times.
- Pellet the RNA by centrifugation at 4°C for 10min in a microcentrifuge.
- Carefully pour off the isopropanol without dislodging the RNA pellet.
- Rinse with 75% EtOH (DEPC H_2O), being careful to not dislodge the pellet. Centrifuge 5min at 4°C
- Remove all of the residual EtOH with a pipet. Air dry 10-15min.
- Resuspend the nucleic acid in 52ul RNAse-free water.

Storage, Quantitation and Determination of Quality of RNA

- Electrophoresis on 1% Agarose gel with 1ul sample.
- Dilute 1ul sample to 100ul with TE(10mM Tris.HCl pH 8, 1mM EDTA), and measure A260 and A280 with a spectrophotometer.. Alternatively 1ul sample can be used to measure A260 and A280 on a Nanodrop ND-1000 Spectrophotometer without dilution.
- Concentration of RNA sample = 40 x A260 x 100 (Dilution factor) (ug/ml)
- A260/A280 Ratio = A260/A280, ranging from 1.7 to 2.1
- Add 100ul EtOH (2 volume) and 5ul 3M NaOAC (1/10 volume), store at -20°C

Regents

- RNAProtect Bacteria Reagent Qiagen
- MasterPure RNA Isolation Kit Epicentre

Note: This protocol was adapted from the original Qiagen and Epicentre protocols.