Icahn School of Medicine at Mount Sinai LINCS Center for Drug Toxicity Signatures

Standard Operating Procedure:
Total RNA Isolation

DToxS SOP Index: A-1.0
Last Revision: May 7, 2015
Written By: Evren U. Azeloglu and Gomathi Jayaraman

Approvals (Date):	Joseph Goldfarb	(6/30/2015)
	Marc Birtwistle	(5/5/2015)
	Eric Sobie	(6/30/2015)
	Ravi Iyengar	(5/6/2015)

Quality Control (QC) steps are indicated with green highlight.

Metadata recording is highlighted with yellow highlight and superscript indices.

---

1. Prepare RNAse-free "DEPC water" ahead of time
    a. Add 1 mL diethyl pyrocarbonate (DEPC; Sigma, Cat: D5758) into 1 L distilled deionized water
    b. Stir overnight
    c. Autoclave and allow to cool to room temperature
2. Thaw cell lysates (as prepared by DToxS SOP CE-4.0) on ice and mix by pipetting up and down a few times using a 1000 µL micropipettor
3. Centrifuge at 12,000g for 10 minutes at 4ºC
    a. For this and all future centrifugation steps, be consistent with the directionality of the tubes during spinning. Pellets in most steps will be fragile and faint; keeping the location of pellets consistent is important.
4. Transfer the supernatant into two new 1.5 mL tubes with a 1000 µL micropipette; discard the old tubes with pellets
5. Add 200 µL chloroform (Sigma, Cat: C2432) into each tube and shake vigorously for 1 minute
    a. You can shake vertically by hand or use a high a vortex mixer with Eppendorf inserts at its highest setting
6. Centrifuge at 12,000 g for 15 minutes at 4ºC
    a. This step separates solubilized RNA from insoluble protein and DNA
7. Transfer the supernatants using 200 µL micropipettes into two new 1.5 mL tubes
    a. Pay close attention not to contact, disturb or aspirate any of the interface; any mistake at this step will lead to DNA or protein contamination

    b. The leftover interface contains DNA and protein, which can be isolated separately. Save this for subsequent isolation (DToxS SOP A-2.0) or return to -80ºC storage.
8. Add 500 µL of isopropanol (Sigma, Cat: I9516) into each tube and mix by inverting slowly 5 times
    a. Wait for 10 minutes
9. Centrifuge at 12,000 g for 10 minutes at 4ºC
10. Carefully remove the supernatant from each tube using a 1000 µL micropipette, leaving the intact RNA pellet
11. Wash eachRNA pellet with 1 mL of 75% room temperature ethanol
    a. Prepare 75% ethanol by diluting 100% ethanol (Sigma, Cat: E7023) with DEPC water
    b. Centrifuge at 7,500g for 10 minutes at 4ºC
    c. Carefully remove the supernatant from each tube using 1000 µL micropipette, leaving the intact RNA pellet
12. Repeat Step 11, removing as much ethanol as possible
13. Air dry pellet at room temperature
    a. Leave tubes uncapped under a chemical hood
    b. This process takes approximately 15 minutes if pellets are aspirated completely
14. Combine RNA from the two tubes with 30 µL of RNase-free water (Invitrogen, Cat: 10977-023)
    a. Pipette 30 µL of water into first tube and resuspend by pipetting up and down a few times slowly
    b. Transfer this solution into the second tube and resuspend the second RNA pellet by pipetting up and down a few times slowly
15. Aliquot 5 µL for concentration measurement and QA/QC assays (DToxS SOP A-3.0)
16. Freeze 25 µL RNA stock at -80ºC