

RNA Extraction Protocol

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Tissue Harvest

****Whatever the method of storage when harvesting, it is CRUCIAL that the tissue be stored immediately following sacrifice and extraction!!**

Snap Freezing in Liquid Nitrogen:

- 1) Following immersion, keep the tissue in the Nitrogen until the procedure is completed.
- 2) Upon completion of the harvest procedure, transfer the tissues to empty falcon tubes stored on dry ice.
- 3) Keep the tissue frozen until the homogenization procedure is ready to be performed.

Immersion in RNA Later:

- 1) Upon extraction from the animal, immediately slice the tissue into pieces no wider than **0.5cm** and drop into RNA Later. (The volume of RNA Later should be at least ten times the volume of tissue)
- 2) Store the tissue (until homogenization) according to the following: Initially - overnight at 2-8 C, Then – indefinitely < -20 C, up to four weeks at 2-8 C, up to 7 days at 2-8 C, up to 1 day at 37 C.

*For cultures of cells, pellet out of growth media, wash 3 X PBS, and resuspend in RNA Later.

(Do Not Freeze!)

Homogenization

- 1) For tissues that are snap frozen or slightly in excess, the homogenization of the tissue should be done by mortar and pestle (cooled to temp in a liquid nitrogen bath).
- 2) At the same time, transfer at least 1mL TRIZOL / 100mg tissue to be homogenized into a falcon tube
- 3) Transfer the tissue to the pestle and grind until a layer of very fine dust is all that is left.
- 4) Use an RNase free spatula to transfer the dust to the TRIZOL solution. Be sure to get as much dust as possible.
- 5) Vortex mixture thoroughly.

*For tissues that are very small or highly precious, a hand-held tissue grinder is recommended. The homogenization is performed in the presence of the 1mL TRIZOL / 100mg tissue until the tissue is completely dissolved in solution.

*For cultures of cells (suspended in solution), quantify, pellet the cells, and resuspend in TRIZOL at a volume of 5×10^6 cells / 1mL TRIZOL.

- 6) Once homogenized, aliquot the solution to eppendorf tubes and leave in TRIZOL at room temp for five minutes.

Phase Separation

- 7) Add 200ul chloroform / 1mL TRIZOL (originally used), vortex for 15 seconds, and leave at room temp for 2-3 minutes.
- 8) Centrifuge samples at 12,000g for 15 minutes at 2-8 C.

RNA Precipitation

- 9) Following centrifugation, there will be three phases visible within the tube. Transfer the aqueous phase (top) to a fresh tube, being careful not to contaminate the solution with the other phases. Contamination will be obvious by the presence of any flakes or unclear liquid.
- 10) Add 500ul isopropanol / 1mL TRIZOL (originally used) to the new tube and incubate at room temp for 10 minutes.
- 11) Centrifuge samples at 12,000g for 10 minutes at 2-8 C.

RNA Wash and Resuspension

- 12) Following centrifugation, remove the supernatant.
- 13) Wash RNA pellet with 80% EtOH / 1ml TRIZOL (originally used) and vortex.
- 14) Centrifuge samples at 7,500g for 5 minutes at 2-8 C.
- 15) Remove supernatant. Allow remaining EtOH to air dry for 2-3 minutes.
- 16) Transfer tubes to 70 C heat block and let sit for 2-3 minutes.
- 17) Redissolve the pellet in 81ul of DEPC water.

Dnase Treatment (Using Ambion's Dnase Treatment Kit)

- 18) Add 8ul of 10X DNase I Buffer.
- 19) Add 2ul of DNase I Enzyme.
- 20) Vortex, quick spin and incubate at 42 C for 25 minutes.

Rneasy Column Purification (Using Qiagen's Rneasy Protocol)

- 21) Add 350ul Buffer RLT (with BME-10ul/ml Buffer RLT).
- 22) Add 250ul 100% EtOH.
- 23) Apply entire volume to RNeasy column and spin full speed for 1 minute.
- 24) Reapply entire volume to RNeasy column and spin full speed for 1 minute.
- 25) Transfer column to new 2ml collection tube.
- 26) Add 750ul Buffer RPE and spin full speed for 1 minute.
- 27) Discard flow-through and add 750ul Buffer RPE and spin full speed for 1 minute.
- 28) Discard flow-through and spin full speed for 1 minute.
- 29) Transfer column to new labeled 1.5ml Eppendorf tube.
- 30) Add 56ul DEPC H₂O and let sit for 2 minutes.
- 31) Spin at full speed for 2 minutes.
- 32) Discard column and transfer tube to ice.

Quantification and Quality Control

- 33) Quantify each sample using Nanodrop.
- 34) Run 5ul of each sample on Agarose gel.