

## RNA Isolation - cDNA Procedures

### *Precautions -*

- 1. Trizol is toxic - causes burns with skin contact.*
- 2. Always wear gloves.*
- 3. Trizol - work in a fume hood- avoid breathing fumes.*
- 2. Avoid introducing RNAses into the samples:*
  - a. Wear gloves.*
  - b. Use only new/sterilized plastic ware/instruments that are reserved for RNA work.*
  - c. Do not touch anything that will come into contact with the samples with your bare hands.*
  - d. Soak all non-disposable items for 10 minutes in 0.5 M NaOH, rinse with glass distilled water and autoclave.*

### RNA Isolation

#### **Preparation:**

- 1. Soak reusable items in 0.5 M NaOH for 10 minutes. Rinse with distilled water and autoclave.**
  - a. A set of grinders (one for each sample to be isolated).**
  - b. Three tube racks.**
- 2. Label two sets of 1.7 ml tubes, one rack of mixer mill tubes - wear gloves.**
- 3. If samples are in RNALater, remove as much liquid as possible.**
- 4. If samples are frozen, keep them on liquid N<sub>2</sub> until they are homogenized.**
- 5. Reagents:**
  - Trizol
  - Chloroform
  - Isopropyl alcohol
  - High-salt precipitation solution (0.8 M Sodium Citrate plus 1.2 M NaCl)
  - 60% Ethanol
  - Sodium citrate storage buffer (1 mM, pH 6.4)

#### **Procedure:**

##### **1. Homogenize tissue.**

Leaf tissue should be crushed into the bottom of tubes prior to processing.

Set up rack of tubes for the mixer mill.

- Place in a tube with 875  $\mu$ L Trizol plus 125  $\mu$ L RNA Isolation aid (Ambion)
- Add one tungsten-carbide ball to each tube.
- Mix in mill for 90 s at 300 hz.
- Rotate racks and mix in mill for an additional 90 s at 300 hz.
- Incubate 15 minutes in oven at 25 °C.

##### **2. Remove solids.**

- Centrifuge tube racks for 15 minutes at 3,700 rpm on the rack centrifuge
- Remove supernatant with a pipette and transfer to new 1.7 ml tubes (2 x 400  $\mu$ L)

##### **3. Phase separation (\* store at -70 °C).**

- add 200  $\mu$ L of chloroform to each tube.
- Vortex vigorously for 3 x 10 s and incubate an additional 3 minutes at 25 °C.
- Centrifuge @ 9,000 rpm for 15 minutes.

#### **4. RNA precipitation.**

- Transfer aqueous (upper) layer (2 x 200  $\mu$ L) to a new tube (do not disturb interface).
- Add 250  $\mu$ L isopropyl alcohol (2-Propynol).
- Add 250  $\mu$ L of high-salt precipitation solution.
- Incubate 12 h at -20  $^{\circ}$ C.

#### **5. RNA wash and resuspension.**

- Prepare resuspension solution:
  - Add 50  $\mu$ L of Sodium Citrate storage buffer per sample.
  - Add RNAsecure reagent to dilute to 1X (volume = total volume/25).
  - Incubate solution at 60  $^{\circ}$ C for 10 minutes.
- Centrifuge @ 10,500 rpm for 30 minutes.
- Pour off supernatant remove as much as possible with a P200 pipettor.
- add 1000  $\mu$ L of 60% ethanol (\* **store at -20  $^{\circ}$ C**).
- Vortex and centrifuge @ 10,500 rpm for 20 minutes.
- Pour off supernatant.
- Centrifuge @ 10,500 rpm for 1 minute.
- Remove residual supernatant with a P10.
- Air dry samples for 5 - 10 minutes- until the ethanol has evaporated.
- Add 50  $\mu$ L of Sodium Citrate storage buffer.
- Incubate for 10 minutes at 55  $^{\circ}$ C.

#### **6. Determine concentration/purity.**

- dilute 1 - 5  $\mu$ L RNA sample in storage buffer volume to 500  $\mu$ L.
- Determine absorbance at 260 nm and 280 nm.
- The ratio absorbance 260/280 should be > 1.6.
- Concentration is absorbance 260 \* 40 \* (500/sample volume)
- **Store at -70  $^{\circ}$ C (split into several smaller tubes).**