**RNA Extraction from Kumar et al. 2007**

**Materials**

* **Reagents**
  + Buffer (3X), pH: 9.6
    - 0.3 M Tris
    - 0.6 M NaCl
    - 3% SDS
    - Dilute 1:3 with MiliQ water
    - Add Fresh
      * 0.65% Na sulfite
      * 2% ß-mercaptoethanol

|  |  |  |  |
| --- | --- | --- | --- |
| **CONCENTRATION** | **CONVERSION** |  | **g/1000 mL** |
| 0.3 M Tris | 0.3M = 0.3 moles/L  1L \* 0.3 moles/L = 0.3 moles  0.3 moles \* 121.14 g/mole = **36.34 g/L** |  | **36.34 g/L** |
| 0.6 M NaCl | 0.6M = 0.6 moles/L  1L \* 0.6 moles/L = 0.6 moles  0.6 moles \* 58.4 g/mole = **35.04 g/L** |  | **35.04 g/L** |
| 3% SDS | 0.03 \* 100 ml =  3g/100ml\* 1000ml/L = **30 g/L** |  | **30 g/L** |
| Adjust pH to 9.6:  Using NaOH if using Tris  Using Hydrochloric acid if using tris base | | | |
| Water |  |  | **Fill to 1 L** |

* For a 1200ml solution
* For a 1:3 dilution:
  + Add 400 ml of 3x buffer to 800 ml of MiliQ water
* For 0.65% (w/v) Na sulfite:
  + 100 \* 0.0065 = 0.65 g/100ml
  + 0.65g/100ml \*1000ml/L = 6.5 g/L
  + 6.5 g/L = 6.5 g/1000ml = n/1200 ml
    - 7800 = 1000n
    - 7.8 g/1200 ml
* For 2% (v/v) ß-mercaptoethanol
  + 100 \* 0.02 = 2 ml/100ml
  + 2 ml/100ml \* 1000 ml/L = 20 ml/L
  + 20 ml/L = 20 ml/1000ml = n/1200 ml
    - 24000 = 1000n
    - 24 ml/1200 ml
  + Phenol (Water Saturated)
  + Chloroform-isoamyl (24:1)
  + 3 M NaAOc pH 5.2
    - 3 M = 3 moles/L
    - 1L \* 3 moles/L = 3 moles
    - 3 moles \* 82.03 g/mole = 246.09 g/L
      * Dissolve 246.1g of NaAOc in 500 ml of MiliQ water
      * Adjust pH to 5.2 with glacial acetic acid
      * Let solution cool overnight
      * Again, adjust pH to 5.2 with glacial acetic acid
      * Adjust volume to 1 L
      * Filter sterilize
  + Absolute ethanol
  + 70% ethanol
  + DEPC H20/nuclease free water
  + DNase
* **Equipment**
  + Centrifuge with 14,000 g capacity
  + Vacufuge
  + Micro-pipettes
* **Consumables**
  + 2 ml microcentrifuge tubes 5X or 50 ml centrifuge tubes
  + Mico-pipette tips

**Method**

1. Grind ~ 200 mg of sample tissue
   1. (200 mg sample/1.5 ml buffer = n mg sample/x ml buffer)
   2. Deliver 1.5 ml of buffer to 2 ml microcentrifuge tubes
   3. Add ~200 mg of sample from step 1 to tube with 1.5 ml of buffer from step b
   4. Invert tubes
   5. Centrifuge tubes at 14,000 g for 5 minutes
2. Transfer supernatant to new 2 ml microcentrifuge tube
   1. Discard precipitate
   2. Invert tubes with supernatant
   3. Centrifuge tubes at 14,000 g for 5 minutes
3. Transfer supernatant to new 2 ml microcentrifuge tube
   1. Discard precipitate
4. If sample volume > 1.4 ml then split sample evenly into two 2 ml microcentrifuge tubes
   1. If sample volume < 1.4 ml then keep in one 2 ml microcentrifuge tube
   2. (e.g. if 1.4 ml then add 700 ul per tube x 2X/sample)
   3. Add equal volume of phenol to each sample
   4. Invert tubes with supernatant
   5. Centrifuge tubes at 14,000 g for 5 minutes
5. Transfer supernatant to new 2 ml microcentrifuge tube
   1. Discard precipitate
6. Add equal volume of chloroform-isoamyl (24:1) to each sample
   1. Invert tubes with supernatant
   2. Centrifuge tubes at 14,000 g for 5 minutes
7. Transfer supernatant to new 2 ml microcentrifuge tube
   1. Discard precipitate
   2. Repeat step 6 and add more chloroform-isoamyl (24:1) to each sample if needed
8. Add 1/10 of total volume of 3 M NaAOc pH 5.2 to each sample
9. Add 2X of absolute ethanol to each sample
10. Incubate at 20°C overnight
11. Centrifuge tubes at 14,000 g for 15 minutes
12. Decant
13. Wash pellet 3x with cold 70% ethanol
    1. (fill tube to rim with each rinse to wash remaining NaAOc).
14. Vacufuge tubes at 14,000 g for ~5 minutes
15. Add 50 ul of DEPC H20/nuclease free water
16. Treat with DNase
    1. For 50 ul solution:
       1. 30 ul of 10 ug RNA solution
       2. 5 ul of DNase Buffer
       3. ~1 ul of 2U DNase per 1 ug DNA present
       4. 14 ul of nuclease-free water