

## RNA preparation from Cells in culture

- 1) Always wear gloves
- 2) Always on ice
- 3) Rinse cells twice with PBS (10mls/100cm plate)
- 4) Add 1ml RNazol per 60 or 100cm/dish
- 5) Scrape cells immediately and lyse by vigorously pipetting through a 1000ul tip until homogeneous. Put into a 5ml falcon tube (#2063). Add additional 1ml of RNazol to plate and repeat to rinse.
- 6) Add 0.2ml Chloroform (per 2ml RNazol) to the homogenate.
- 7) Vortex for 15 seconds
- 8) Ice 5 minutes
- 9) Spin in High speed centrifuge at 10,500rpm (12,000g) 4C for 15minutes
- 10) Transfer top aqueous phase (leave interface behind) to a fresh 5ml falcon tube
- 11) Add equal volume (1ml) of chloroform
- 12) Vortex for 15 seconds
- 13) Ice for 5 minutes
- 14) Spin in High speed centrifuge at 10,500rpm (12,000g) 4C for 15minutes
  - a. Use blue adapters with a kimwipe stuck at the bottom so that the lids do not fly off of the 5ml falcon tube
- 15) Transfer top aqueous phase to a clean 5ml falcon
- 16) Add equal volume (1ml) isopropanol
- 17) Vortex
- 18) Incubate overnight at 4C
- 19) Spin in High speed centrifuge at 10,500rpm (12,000g) 4C for 15minutes
- 20) Pour off supernatant
- 21) Wash pellet by adding 500ul 75% cold ethanol and transfer to a microfuge tube
- 22) Spin in microfuge at high speed in cold room
- 23) Pipet off all ethanol. Important to pipet off ALL that you can so that it can dry easier.
- 24) Let air dry. Should only take 5-10 minutes. No moisture should be left but it is important **not to over dry**. As soon as the ring of moisture around the pellet is gone, go to next step.
- 25) Resuspend the pellet in approx 25ul of DEPC-treated water
- 26) Read OD with 260 and 280
- 27) Store at -80C

### DEPC-treated water

0.1% DEPC in water

Autoclave to inactivate the DEPC