Jenny DuRose -- Updated 08-02-07

DNase Treatment of Total RNA:

Incubate total RNA with DNase at 37°C for 30min.

RNA	up to 30µg
5X DNase Buffer	4µL
DNase (Promega M6101)	2µL
RNaseOUT (Invitrogen 10777-019)	1µL
RNA in H_2O	to 20µL

After 30min incubation, add 200µL urea buffer and 250µL Phenol/chloroform/isoamyl alcohol (25:24:1 Rouche 101003 or 25:24:1 Fisher BP 1752^{l} 400).

Vortex and centrifuge at 14k rpm @ RT for 5min.

Isolate the aqueous (top) layer, and ethanol precipitate. (can add glycogen)

Add 700µL 100% EtOH and 1µL RNase free glycogen (Fermentas R0561). Incubate at –20-80°C for at least 20min. Centrifuge at 14K rpm at 4°C for 30min. Completely remove ethanol, resuspend RNA pellet in 21µL RNase free H_2O. Repeat DNase digestion one more time, the volume of the reaction can be reduced to 30µL, if desired.

5X DNase Digestion Buffer	**1X**	**stock**	**10mL**
250mM Tris HCl pH7.4	50mM	1M (20X)	2.5mL
50mM DTT	10mM	1M (100X)	500µL
15mM $MgCl_2$	3mM	1M (33.3X)	150µL
RNase free H_2O			6.85mL

Urea Buffer	**10mL**
Urea	4.2g
2X Urea (-) Buffer	5mL
RNase Free H_2O	2.5mL

2X Urea (-) Buffer	**1X**	**stock**	**50mL**
20mM Tris HCl pH 7.4	10mM	1M	1mL
20mM EDTA pH 8.0	10mM	0.5M	2mL
700mM NaCl	350mM	5M	7mL
2% SDS	1%	10%	10mL
RNase Free H_2O			30mL

RNase Free H_2O
Make 0.1% diethyl pyrocarbonate (DEPC) solution with ddH_2O in fume hood. Stir for 30min to inactivate RNase. Autoclave for 30min to remove DEPC.

RNase Free 1M Tris HCl pH 7.4
Dissolve 60.57g RNase Free Tris base (121.14g/mol) in 400mL ddH_2O.
pH to 7.4 using concentrated HCl. Add ddH_2O to 500mL. In fume hood add 5mL diethyl pyrocarbonate (DEPC) and stir for 30min to inactivate RNase. Autoclave for 30min to remove DEPC.
(Tris inactivates DEPC so 1% solution in used instead of 0.1%)

RNase Free 0.5M EDTA pH 7.4
Dissolve 73.06g disodium EDTA (292.25g/mol) in 300mL ddH_2O.
pH to 8.0 using 10N NaOH. Add ddH_2O to 500mL. In fume hood add 500µL diethyl pyrocarbonate (DEPC) and stir for 30min to inactivate RNase. Autoclave for 30min to remove DEPC.

RNase Free 5M NaCl
Dissolve 146.1g NaCl (58.44g/mol) in 500mL ddH_2O. In fume hood add 500µL diethyl pyrocarbonate (DEPC) and stir for 30min to inactivate RNase. Autoclave for 30min to remove DEPC.

RNase Free 1M $MgCl_2$
Dissolve 40.66g $MgCl_2$ (203.30g/mol) in 200mL ddH_2O. In fume hood add 200µL diethyl pyrocarbonate (DEPC) and stir for 30min to inactivate RNase. Autoclave for 30min to remove DEPC.

RNase Free DTT
Dissolve RNase Free DTT in RNase Free H_2O (see above).
DO NOT AUTOCLAVE!

RNase Free 10% SDS
Dissolve RNase Free SDS in RNase Free H_2O (see above).
DO NOT AUTOCLAVE!