

DNase Treatment of Total RNA:

Hazards to be aware of:

- Phenol/Chloroform/Isoamyl alcohol, possible carcinogen and corrosive.
- DEPC, toxic may form urethane when mixed with ammonia.

*****PLEASE REFER TO MSDS SHEETS AT END OF PROTOCOL FOR DIRECTIONS ON CLEAN UP IF A SPILL SHOULD OCCUR, AND APPROPRIATE FIRST AID MEASURES*****

PPE:

- **Gloves**
- **Safety glasses**
- **Lab Coat**
- **Fume Hood (for all work with DEPC and Phenol Chloroform)**

Protocol

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

(Use up to 30µg of total RNA)

	Rxn 1	Rxn 2
5X DNase Buffer	16µL	6µL
DNase (Promega M6101)	3µL	2µL
RNaseOUT (Invitrogen 10777-019)	1µL	1µL
RNA in H ₂ O	60µL	21µL

If DNase Digestion Buffer is not fresh, supplement with 1.75µL 100mM DTT (1.5mM final conc).

After 30min incubation, add 200µL urea buffer and 250µL Phenol/chloroform/isoamyl alcohol (25:24:1 Rouche # 101003) (or 25:24:1 Fisher BP 1752¹ 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 $21\mu\text{L}$ RNase free H_2O . Repeat DNase digestion one more time, the volume of the reaction can be reduced to $30\mu\text{L}$, if desired.

5X DNase Digestion Buffer ***Use RNase free stock solutions***

1M Tris HCl pH7.4	2.5mL
1M DTT	$500\mu\text{L}$
1M MgCl_2	$150\mu\text{L}$
RNase free H_2O	6.85mL

Urea Buffer

Urea	4.2g
2X Urea (-) Buffer	5mL
bring up to 10mL with RNase Free H_2O	

2X Urea (-) Buffer ***Use RNase free stock solutions***

1M Tris HCl pH 7.4	4mL
0.5M EDTA pH 8.0	8mL
5M NaCl	28mL
10% SDS	40mL
<u>RNase Free H_2O</u>	<u>120mL</u>
Total	200mL

RNase Free H_2O

Make 0.1% diethyl pyrocarbonate (DEPC) solution with dd H_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 dd H_2O . pH to 7.4 using concentrated HCl. Add dd H_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 is used instead of 0.1%)

RNase Free 0.5M EDTA pH 7.4

Dissolve 73.06g disodium EDTA (292.25g/mol) in 300mL ddH₂O. pH to 8.0 using 10N NaOH. Add ddH₂O 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 ddH₂O. In fume hood add 500μL diethyl pyrocarbonate (DEPC) and stir for 30min to inactivate RNase. Autoclave for 30min to remove DEPC.

RNase Free 1M MgCl₂

Dissolve 40.66g MgCl₂ (203.30g/mol) in 200mL ddH₂O. 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 H₂O (see above).
DO NOT AUTOCLAVE!

RNase Free 10% SDS

Dissolve RNase Free SDS in RNase Free H₂O (see above).