

## **DNase treatment of RNA**

### **Sample preparation**

**Dr Alison Richmond**  
**Post doc**

[a.richmond@rowett.ac.uk](mailto:a.richmond@rowett.ac.uk)

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**We used the NuGO Standard Operating Procedure (SOP) number 51 produced by the University of Aberdeen. Details of the SOP are available via the web link:**

<http://www.nugo.org/frames.asp?actionID=38661&action=loginFromPP>

### **Introduction**

RNA samples to be used for quantitative real time RT-PCR is routinely subjected to DNase treatment for remove any contaminating DNA. Because an accurate estimation of RNA concentration is very important for the absolute quantification of the mRNA copy numbers, any contamination with DNA will result in an inaccurate quantification.

Make sure to use reagents, tips and tubes that are RNase and DNase free.

### **Equipment/reagents**

1 ml RNase free tubes  
0.5 ml thin walled RNase free PCR tubes  
Sterile RNase free tips of appropriate size  
DEPC treated ddH<sub>2</sub>O  
75% Ethanol (made up using 0.1 % DEPC- ddH<sub>2</sub>O)  
3M Sodium Acetate pH 4.8 (made up using DEPC- ddH<sub>2</sub>O)  
Isopropanol  
Invitrogen DNase I Amplification Grade 100U (1U/μl)  
Nuclease free water (Qiagen, 129114)  
PCR Machine  
Refrigerated centrifuge

### **Reagent preparation**

#### **0.1 % DEPC water**

500 μl DEPC  
500 ml millipore H<sub>2</sub>O

Cover and leave to stir overnight in fume hood. Autoclave next morning and store at 4 °C.

### **3M Sodium Acetate pH 4.8**

24.6 g sodium acetate (Mw: 82.03 g/mol)

Dissolve in approx 20 ml 0.1 % DEPC- ddH<sub>2</sub>O on magnetic stirrer. Adjust pH to 4.8 with glacial acid. Adjust volume to 100 ml with 0.1 % DEPC- ddH<sub>2</sub>O.

Autoclave and dispense into ~ 5ml aliquots

### **75% ethanol**

37.5 ml 99% ethanol + 12.5 ml 0.1 % DEPC- ddH<sub>2</sub>O

### **Method**

1. Determine concentration of RNA by using Nanodrop.
2. To 20µg of RNA add 4µl Buffer and make up to 18µl with DEPC H<sub>2</sub>O into a 500µl Eppendorf and gently mix.
3. Add 2µl DNase and gently flick and pulse spin.
4. Incubate at 25°C for 10 mins. Can use a PCR block and then hold at 4°C, until ready for next step, up to 1 hour max.
5. Place samples on ice and add following:

30µl DEPC H<sub>2</sub>O  
5µl Na Acetate 3M pH 4.8  
55µl Isopropanol

Prepare a master mix if treating a large number of samples and add 90µl of MM to each tube.

6. Mix by inverting and incubate at -20°C for 1-2 hours.
7. Centrifuge at 12, 000 rpm, 15 mins, 4°C.
8. Remove supernatant by pipetting and wash pellet in 500µl 75% EtOH DEPC and centrifuge at 12, 000 rpm, 5 mins, 4°C.
9. Removed EtOH by pipetting and air-dry pellet for ~15 mins on ice. A pulse spin will help to remove residual liquid.

10. Resuspend pellet in 30 $\mu$ l Nuclease free water. Leave pellet to sit in water for 10-15 mins on ice to aid resuspension. Resuspend by pipetting
11. Nanodrop DNase treated samples (2 $\mu$ l + 2 $\mu$ l water).
12. Allow 100 - 300ng for Agilent analysis in ~3 $\mu$ l.
13. Aliquot the samples in RNase/DNase free tubes in appropriate volumes and/or concentrations depending on next stage i.e. micro array analysis, real time PCR. This will help reduce freeze/thaw cycles and RNA denaturation. Store at  $-80^{\circ}\text{C}$ .