

# DNase I Treatment of RNA Samples and Sample Dilution

- 1) Determine the RNA concentration of your samples
- 2) Dilute the total RNA to **27-30 ng/ $\mu$ l** in DEPC-H<sub>2</sub>O
- 3) Prepare the following dilution of RNase-free DNase I (if necessary)

## 1/10 Dilution of RNase-free DNase I (if necessary)

Stock	Volume
H <sub>2</sub> O	8 $\mu$ l
10X PCR Bfr*	1 $\mu$ l (commercial nuclease-free 10X PCR buffer)
RNase-free DNase I (10 U/ $\mu$ l)	1 $\mu$ l
	<hr/> <hr/> 10 $\mu$ l

- 4) Using the following table as a guide, make enough DNase I master mix to add to all your RNA samples + 1 extra sample for overage
- 5) Add the appropriate volume of DNase I master mix to each RNA sample tube

## Use ISC Bioexpress 1.5 ml screw cap tubes, cat #C-3295-2

If you are local, you can pick up the tubes for free from the Core Lab

## Treatment of Total RNA

Stock	Recommended Sample Dilutions for Submission to the QGCL			
Total RNA ( <b>27-30 ng/<math>\mu</math>l</b> )	61.4 $\mu$ l	118 $\mu$ l	236 $\mu$ l	472 $\mu$ l
50 mM MgCl <sub>2</sub> (1 mM final)	1.3 $\mu$ l	2.5 $\mu$ l	5 $\mu$ l	10 $\mu$ l
1/10 DNase I (1 U/ $\mu$ l)	1.0 $\mu$ l	2 $\mu$ l	4 $\mu$ l	8 $\mu$ l
Final Volume	65.0 $\mu$ l	125 $\mu$ l	250 $\mu$ l	500 $\mu$ l

At 4  $\mu$ l/well of sample, each assay will use  $\approx$ 18-20  $\mu$ l of sample volume

This larger volume is due to overages required for accurate pipetting by the robots

Final RNA concentration should be  $\approx$  25 ng/ $\mu$ l = 100 ng RNA/well

- 6) Incubate the tubes at 37°C for 30 minutes
- 7) Immediately transfer the tubes to 75°C for 10 min (exactly) to kill the DNase I;  
put sample tubes on ice immediately after heating
- 8) Store the treated RNA at -80°C (good for at least 3 years)

\* any commercial nuclease-free 10X PCR buffer

## Standard DNase I procedure from the Quantitative Genomics Core

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