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High Capacity cDNA Reverse Transcription Kit Protocol

Most of these details can be verified on Thermo website, check if questions.

0. RNaseZap all tube racks, gloves, and pipettes. Spray down the bench paper and use proper RNA techniques throughout. Keep RT enzyme and samples on ice.
1. Thaw all kit reagents (except the enzyme) to room temperature. Thaw RNA samples on ice.
2. Dilute RNA samples to a common concentration to equal 10ul. Be sure to use dH₂O or RNase-Free H₂O. Prepare this in either a strip tube with covers or a large 96 well plate.
 - a. I have shared a file on Dropbox. The left side of the red line is where you change your values, and you will dilute all samples to the lowest value of [RNA]. The values on the right of the red line will auto populate and explain clearly how to make the proper solution of RNA. This file is called “[RNA].xlsx” and it’s in Hughes_Lab_Shared_Folder > Protocols > cDNA or Reverse Transcription or qPCR.
3. After kit reagents are liquid, briefly vortex and gently spin down.
4. Create Master Mix of reagents for a total of 10ul per sample, accounting for some pipetting loss:

Reagent	Volume		x	SAMPLES =	ul
dH ₂ O	4.2 ul				ul
10X RT Buffer	2.0 ul				ul
Random Primers	2.0 ul				ul
dNTPs	0.8 ul				ul
RT Enzyme (add last)	1.0 ul				ul
TOTAL	10 ul				

5. Pipette 10ul of the Master Mix into each well of RNA in the well plate or tube strip.
6. Place on the thermocycler and run the program called “cDNA RT for qPCR ABI” from 17 September 2019. If you want to double check you have the correct temperatures and times, you can just check this information on the Thermo website.

	Step 1	Step 2	Step 3	Step 4
Temperature (°C)	25°	37°	85°	4°
Time	10 minutes	120 minutes	5 minutes	Hold