

cDNA preparation

- using Gibco-Brl Superscript II RNase H- reverse transcriptase and oligo dT primers at 500 ng/ μ l
- Prepare cDNA from total RNA – between 1 μ g-0.5 μ g (if making for Q-PCR, will dilute by 1:20 for this range of RNA; dilute less if using less RNA).

SAMPLES:

1. Prepare Mix I on ice

Reagent	Amt for 1 rxn	Amount for ___ rxns
Oligo dT	1 μ l	
10 mM dNTP mix (10 mM each dATP, dCTP, dGTP, dTTP)	1 μ l	
DEPC H2O	Bring up to total volume of 12 μ l total, if necessary	typically don't use because RNA is diluted to 100 ng/ μ l

2. aliquot 2 or more μ l of Mix I to appropriate # of tubes and then add RNA to each
3. Incubate at 65° C – 5 minutes and transfer immediately to ice
4. Prepare Mix II:

Reagent	Amount for 1 rxn	Amount for ___ rxns
5X first strand buffer	4 μ l	
0.1M DTT	2 μ l	
RNase Out	1 μ l	

5. Add 7 μ l Mix II to each tube
6. Incubate at 42° C – 2 minutes
7. + 1 μ l Superscript II and mix gently by pipetting.
8. Incubate at 42° C 2 hours (can shorten to 1 hour if necessary).
9. Heat to 65° C – 10 minutes to stop reaction.
10. Use directly in PCR reaction. Usually 0.5 μ l is sufficient for moderately abundant message with standard PCR.