cDNA Synthesis (SuperScript II RT)

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BACKGROUND

This protocol is used to synthesis cDNA from isolated RNA using the SuperScript II reverse transcriptase enzyme with the aim to use the synthesized cDNA in subsequent 2-stage RT-qPCR reactions.

MATERIALS

- SuperScript II First-Strand Synthesis System for RT-PCR Kit
- Random pentadecamers (10 µM)
- 10 mM dNTP mix
- RNAse-free water
- 0.1M dithiothreitol (DTT)
- RNAse inhibitor (40 U/µL)

PROTOCOL

1. If not already diluted to 100 ng/µL (±25 ng/µL), use a nanodrop spectrometer to standardize samples.
    a. Aliquot some undiluted RNA into a separate Eppendorf tube as a reserve in the event you over-shoot your dilution.
    b. Dilute in increments, under-shooting the desired concentration so as not to over-dilute.
2. Briefly pulse centrifuge components before use and mix in a sterile PCR tube.

Volume (µL)	Reagent
8	100 ng/mL total RNA (2 ng total)
1	10 mM dNTPs
1	10 µM random pentadecamers

3. Incubate RNA/primer/dNTP mix at 65°C for 5 mins, then place on ice for at least 1 minute.

4. In a separate 1.5 mL tube, prepare the following 2x reaction mix, adding each component in the indicated order. Prepare enough for all samples.

Volume (µL)	Reagent
4	5x FS buffer
2	0.1 M DTT
2	50 mM $MgCl_2$
1	40 U/µL RNase inhibitor

5. Add 9 µL of the 2x reaction mix to each RNA/primer/dNTP mix from step 2.
6. Mix sample by flicking, then briefly centrifuge to spin down and collect the mixture.
7. Let it stand at room temperature for 2 minutes.
8. Add 1 µL of SuperScript II RT to each tube.
    a. Negative control: add 1 µL of nuclease-free water instead of the enzyme.
9. The following steps (10-13) are completed in a thermocycler:
10. Incubate at 25°C for 10 minutes.
11. Incubate at 42°C for 50 minutes.
12. Terminate the reaction by incubating at 70°C for 15 minutes.
13. Chill at 4°C until use (the thermocycler should keep samples at 4°C until samples are removed after cycle completion).
14. Store at -20°C or use for PCR immediately.

NOTES

- https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2FsuperscriptII_pps.pdf
- Kit designed for use with 1-5 microg starting total RNA.
- If DNA contamination is suspected in RNA sample, treat with DNAse-treat prior to applying kit.