1st Strand cDNA Synthesis System for Quantitative RT-PCR

Catalog No. NP100041 (11801-025) 25 Reactions
Catalog No. NP100042 (11801-100) 100 Reactions

Important Information
The product you have received is authorized for laboratory research use only. The product has not been qualified or found safe and effective for any human or animal diagnostic or therapeutic application. Uses other than the labeled intended use may be a violation of applicable law.

Components (store at -20ºC):

	NP100041	NP100042
5X cDNA Synthesis Mix *5X concentrated solution of optimized buffer, magnesium, primers (oligo(dT)$_{20}$ + random primers), and dNTPs*	100 µL	400 µL
Reverse Transcriptase, 50 U/µL *RNase H$^+$, with RNase inhibitor carrier protein*	25 µL	100 µL
Nuclease-free distilled water	1.5 mL	1.5 mL

Instructions:
Thaw all components (except enzyme), mix each component thoroughly, and centrifuge before use. Hold all components on ice before use.

Add the following to a 0.2-mL thin-walled PCR tube or 96-well PCR reaction plate sitting on ice:

RNA (10pg to 1 µg total RNA)		x µL
Nuclease free distilled water	15-x µL	
5X cDNA Synthesis Mix		4.0 µL
Reverse Transcriptase		1.0 µL
Total volume		20.0 µL

Note:When performing multiple cDNA syntheses, a master mix can be prepared with water, 5X cDNA Synthesis Mix and Reverse Transcriptase.

	25 Rxns	100 Rxns
Nuclease free distilled water	250 µL	1.0 mL
5X cDNA Synthesis Mix	100 µL	400 µL
Reverse Transcriptase	25 µL	100 µL
Total volume	375 µL	1.5 mL

Dispense 15 µL of cDNA master mix to each well / tube.
Add 5 uL of RNA sample to each reaction (10pg to 1 µg total RNA).
Cover the reaction plate with sealing film or cap each reaction.

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Instructions (continued):

Vortex gently and centrifuge 10 seconds to collect contents.

Place tube(s) in a thermocycler programmed as follows:
1 cycle: 22ºC, 5 min
1 cycle: 42ºC, 30 min
1 cycle: 85ºC, 5 min
4ºC hold
Initiate run.

After completion of cDNA synthesis, use 1/5th to 1/10th (2 µL to 4 µL) of the first-strand reaction for qPCR amplification.

Note:Dilution of the first-strand reaction with TE buffer [10 mM Tris (pH 8.0), 1 mM EDTA] may be useful when quantifying multiple RNAs in a single cDNA reaction, or when storing the material for future use. Additionally, when performing real-time quantitative RT-PCR, it is generally more convenient and accurate to add higher volumes (5 - 10 µL) of target to each qPCR.
cDNA may be stored at -20ºC.