

cDNA Synthesis Kit



TECHNICAL SPECIFICATIONS

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cDNA Synthesis Kit Product #: MBK-100

Description

Stressgen's StressXpress cDNA Synthesis Kit is designed for efficient first-strand cDNA synthesis from different RNA templates. The kit provides high quality components that allow applications that include cDNA synthesis for real-time PCR and conventional quantitative RT-PCR analysis, construction of cDNA libraries, and synthesis of probes for various hybridization techniques. High quality Ribonuclease inhibitor used in our kit reduces RNase contamination during reaction set-up and our anchored oligo d(T)₁₈ primer is specially designed to minimize truncated cDNAs. The maximum cDNA yield from a single reaction that provides a cost-effective solution for analysing multiple genes (cDNA can be diluted for certain PCR reactions) and choice of primers for poly-A and non poly-A RNA templates are some of the important features of our kit.

1st Strand cDNA Synthesis Protocol

Note: RNase contamination can be avoided using dedicated pipettes, sterile RNase-free tubes and pipette tips. Gloves must be worn at all times.

- Set-up the following reaction mixture in a tube on ice:

Component	Volume
Template RNA: Total RNA OR poly(A) RNA	0.1µg to 5µg 10ng to 500ng
Primer: (Anchored Oligo (dT) ₁₈ primer OR Random Decamer primer OR Gene-specific primer)	1µL
PCR Grade Nuclease-Free Water	To 12µL

- Gently vortex or mix by pipetting up and down, centrifuge briefly for 3-5 seconds. Incubate the mixture at 70°C for 5 minutes to remove any secondary structures. Chill RNA on ice.
- Add the following components in the specified order:

Component	Volume
5X cDNA Reaction Buffer	4µL
dNTP's (10mM)	2µL

- Gently vortex or mix by pipetting up and down, centrifuge briefly. Incubate at 37°C for 5 minutes (at 25°C for 5 minutes if random decamer primer is used).
- Add 2µL of the M-MuMLV RT (3.5U/µL). The enzyme mix also contains 10U/µL Ribonuclease Inhibitor.
- Incubate the mixture at 37°C for 60 minutes.
- Incubate at 70°C for 10 minutes to stop the reaction. Chill on ice. The first strand cDNA synthesized can be used directly for amplification by PCR, or be stored at -20°C.

Alternative Protocol for Processing Large Number of RNA Samples

- Set-up the following reaction mixture in a tube on ice for each RNA sample:

Component	Volume
Template RNA: (Total RNA OR poly(A) RNA OR Specific RNA)	an optimized amount
PCR Grade Nuclease-Free Water	to 11 µL

- Incubate the RNA samples at 75 °C for 5 minutes. Chill on ice for 5 minutes.
- Prepare the following master mix for the number of RNA samples (preferable 1 or 2 extra reactions to allow loss of reaction volume during repeated pipetting).

Component	µL x No. of Rxns	Total Volume
5 X cDNA Reaction Buffer	4 x 10	40µL
Oligo d(T) ₁₈ OR Random Decamer Primer	1 x 10	10µL
M-MuLV RT Enzyme mix	2 x 10	20µL
10mM dNTP	2 x 10	20µL
	Total Volume	90 µL

- Mix by pipetting up and down and add 9µL of the mix to each 11µL of the RNA sample and incubate at 37°C for 1 hour. Incubate at 70°C for 10 minutes to stop the reaction.

The Polymerase Chain Reaction (PCR) process is covered by patents owned by Hoffmann-La Roche Inc.

Rev: 10/08/03

CERTIFICATE OF ANALYSIS

cDNA Synthesis Kit

Product #: MBK-100

Sizes: 100 rxns

Lot#: B310415

Materials Included:	Part#	Component	Volume	Description
	MBK-P1	5X cDNA Reaction Buffer	2mL	250mM Tris-HCl (pH 8.3), 375mM KCl, 50mM DTT and 15mM MgCl ₂
	MBK-P2	Anchored Oligo (dT) ₁₈ Primer	100µL	0.5µg/µL (T) ₁₈ V N V=(V = equimolar G and A ; N = equimolar A, T, G, C)
	MBK-P3	Random Decamer Primer	100µL	0.2µg/µL NNN NNN NNN N (N = equimolar concentration of A, T, G, C)
	MBK-P4	dNTP's Mix	200µL	10 mM each dATP, dCTP, dGTP, and dTTP
	MBK-E1	M-MuLV RT and Ribonuclease Inhibitor Mix	2x 100µL	3.5U/µL Moloney Murine Leukemia Virus reverse transcriptase in 50mM Tris-HCl (pH 8.3), 0.1M NaCl, 1mM EDTA, 5mM DTT, 0.1% Triton X-100, 50% v/v glycerol
	MBK-P6	PCR Grade Nuclease-free Water	2mL	Deionized water

Storage of Kit:

Note:

The components in each kit lot # have been quality assured and warranted in this specific combination only; please do not mix them with components from other kit lot #s



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