Protocol
HB cDNA Synthesis Kit

Catalog Number	Size	Concentration
HB001-0050	50 rxns	200 U/μl

Storage Conditions

Stable for up to 1.5 years at -20°C

Description

Introducing the Helix Biosciences cDNA Synthesis Kit, a cutting-edge solution designed for both research and diagnostic applications. This innovative kit is specifically engineered to meet all of your cDNA synthesis needs, including overcoming the most difficult secondary RNA structures across a wide temperature range. It is the newest addition to our reverse transcription product lineup. The HB cDNA Synthesis Kit features our advanced recombinant M-MLV reverse transcriptase, which offers improved thermostability, processivity, and robustness. With optimal cDNA yields, proprietary site mutations for reduced RNase H activity, and an extended half-life, this versatile reverse transcriptase sets new standards in performance. It not only fulfills routine cDNA synthesis requirements but also delivers exceptional results when working with challenging RNA samples.

Kit Content(s)

HB001-0050	RT Enzyme Mix	100 μl x 1 vial
	5X Reaction Buffer Mix	200 μl x 1 vial
	Oligo $d(T)_{20}$ Primer (50 uM)	50 μl x 1 vial
	Random Hexamer Primer (50 ng/ul)	50 μl x 1 vial
	Nuclease-free water	1 ml x 1 vial

Required materials but not provided

- Vortex or equivalent
- Microcentrifuge
- PCR tubes for your instruments
- Ice water bath
- Temperature-controlled water bath or heat blocks; the thermal cycler can also be used.

Template

It is important to avoid cross-contamination between DNA and total RNA, synthetic RNA transcript, poly(A)+mRNA, or any other RNA samples.

Primer Selection

For efficient cDNA synthesis, the recommended primer amounts are as follows:

- 2.5 uM of oligo(dT) to anneal to the 3'-poly(A)+mRNA.
- 2.5 ng/ul of random primers to anneal at non-specific sites of RNA templates.
- 2 uM of gene-specific primers per 20 ul reaction.

Reaction Setup

To perform the cDNA synthesis reaction, follow these steps:

1. In a PCR tube, assemble the following components for each 20 ul reaction: RNA template, primer, and 5X Sharp Reaction Mix. Keep the tube on ice until ready for use.

Component	Final conc.	Volume
RNA template*	10 pg-2 ug total RNA or 10 pg-500 ng mRNA	X ul
5X Sharp Reaction Mix (including dNTPs, $MgCl_2$)*		4 ul
Primers		1 ul
RScript Enzyme Mix	200 U	2 ul
RNase Inhibitor	20-40 U	1 ul
Nuclease-Free Water		Add to 20 ul
Total volume		20 ul

2. Pre-mix the reaction mix and heat it at 65°C for 5 minutes in advance. After pre-heating the RNA on an ice bath for at least 1 minute, add the other components according to the provided table. Gently mix the reaction solution by pipetting.
3. Cap the tubes and place them in a temperature-controlled water bath or heat block. Incubate the tubes at 55°C for 50 minutes for the extension step. The optimal temperature range for extension may be between 42°C and 60°C.
4. After Step 3, incubate the reaction tube at 70°C for 15 minutes to inactivate the Reverse Transcriptase before proceeding with amplification.

Storage Buffer

The enzyme is supplied in a storage buffer containing 20 mM Tris-HCl (pH 7.4), 0.1 M NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01% (v/v) NP-40, and 50% (v/v) glycerol.

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