Protocol
HB Reverse Transcriptase

Catalog Number	Size	Concentration
HBRT001-0250	250 rxns	200 U/µl
HBRT001-0050	50 rxns	200 U/µl

Storage Conditions

Stable for up to 2 years at -20°C

Description

HB Reverse Transcriptase is a ground-breaking innovation engineered to address the needs of both research and diagnostic applications, providing a solution for cDNA synthesis and the intricate task of overcoming highly complex RNA structures across a wide temperature range. This next-generation recombinant M-MLV reverse transcriptase offers improved thermostability, processivity, robustness, and optimal cDNA yields. It features proprietary site mutations to reduce RNase H activity, resulting in an extended half-life. This versatile reverse transcriptase is designed to meet routine cDNA synthesis requirements and deliver exceptional performance, even when faced with the most challenging RNA samples.

Kit Content(s)

HBRT001-0250	HB Reverse Transcriptase	250 µl x 1 vial
	5X Sharp reaction buffer	200 µl x 5 vials
HBRT001-0050	HB Reverse Transcriptase	50 µl x 1 vial
	5X Sharp reaction buffer	200 µl x 1 vial

Required materials but not provided

- Vortex mixer or similar
- Small centrifuge for microtubes
- PCR tubes suitable for your equipment
- Cold water bath with ice
- Temperature-controlled water bath or heat blocks; the thermal cycler can also be utilized.

Template

Avoid cross-contamination with DNA when handling total RNA, synthetic RNA transcripts, or poly(A)+mRNA.

Primer Selection

For optimal cDNA synthesis, use the following primer amounts: 0.5ug of oligo(dT) (3' -poly(A) + mRNA), random primers (for non-specific RNA template annealing), or 2 uM of gene-specific primers per 20 ul reaction.

Reaction Setup

cDNA Synthesis

1. Prepare the following components in a PCR tube for a 20 ul cDNA synthesis reaction. Keep it chilled on ice until just before use.

Component	Final conc.	Volume
RNA template*	10 pg-2 ug total RNA or 10 pg-500 ng mRNA	X ul
5X Sharp reaction buffer (including $MgCl_2$)*		4 ul
Primers		1 ul
HB Reverse Transcriptase	200 U	1 ul
RNase Inhibitor	20-40 U	1 ul
dNTPs (10 mM)	0.2 mM	0.4 ul
Nuclease-Free Water		Add to 20 ul
Total volume		20 ul

*Pre-mix the RNA template, primers, and 5X Sharp reaction buffer, and then heat the mixture at 65°C for 5 minutes beforehand. After pre-heating the RNA and incubating it on ice for at least 1 minute, add the other components as specified in the table.

2. Gently mix the reaction solution by pipetting.
3. Seal the tubes and place them in a temperature-controlled water bath or heat blocks. Incubate the tubes at 55°C for 50 minutes for the extension step. The temperature range of 42°C to 60°C may be optimal for extension.
4. Incubate the reaction tubes from Step 3 at 70°C for 15 minutes to inactivate the Reverse Transcriptase before amplification.

Storage Buffer

The enzyme is provided 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.