Manual

MMLV High Performance Reverse Transcriptase

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MMLV High Performance Reverse Transcriptase is part of the Epicentre™ product line, known for its unique genomics kits, enzymes, and reagents which offer high quality and reliable performance.

BIOSEARCH™ TECHNOLOGIES

GENOMIC ANALYSIS BY LGC

Manual

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1. Introduction

MMLV High Performance Reverse Transcriptase (MMLV HP RT) is highly efficient at producing full-length cDNA from RRNA Templates at least 15 kb. MMLV HP RT demonstrates significantly more activity than other MMLV Reverse transcriptase enzymes. It is capable of producing cDNA from as little as 100 pg of total RNA for real-time RT-PCR (qRT-PCR) analysis and other applications.

MMLV HP RT is supplied at a concentration of 200 units/µL. An MMLV HP RT 10X Reaction Buffer that provides for optimal activity and 100 mM dithiothreitiol (DTT) are also provided.

2. Product designations and kit components

Product	Kit size	Catalogue number	Reagent description	Part number	Volume
MMLV High Performance Reverse Transcriptase	25,000 units	RT80125K	MMLV High Performance Reverse Transcriptase (200 units/µL)	E0115-200D	125 µL
			MMLV HP RT 10X Reaction Buffer	SS000737-D3	600 µL
			DTT (100 mM)	SS000065-D8	600 µL

3. Product specifications

Storage: Store only at -20 °C in a freezer without a defrost cycle.

Storage buffer: MMLV HP RT is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM sodium chloride, 1 mM DTT, 0.1 mM EDTA and 0.1% Triton® X-100 (Rohm & Haas).

Unit definition: One unit catalyzes the incorporation of 1 nmol of dTTP into acid-insoluble material in 10 minutes at 37 °C using saturating amounts of oligo(dT)-primed poly(rA) as template.

Quality control: MMLV HP RT is function-tested in a control reaction using an oligo(dT) primer. In this reaction, the enzyme converts 200 ng of ~2 kb poly(A) RNA into full-length cDNA in 30 minutes or less at 37 °C.

Contaminating activity assays: All MMLV HP RT components are free of detectable contaminating DNA exonuclease and endonuclease and RNase activities.

4. General considerations for cDNA synthesis

Maintaining an RNase-free environment

Ribonuclease contamination is a significant concern for those working with RNA. The ubiquitous RNase A is a highly stable and active ribonuclease that can contaminate any lab environment and is present on human skin. However, creating an RNase-free work environment and maintaining RNase-free solutions is critical for performing successful cDNA synthesis reactions. Therefore, we strongly recommend that the user:

1) Autoclave all tubes and pipette tips that will be used in the cDNA synthesis reactions.
2) Always wear gloves when handling samples containing RNA. Change gloves frequently especially after touching potential sources of RNase contamination such as door knobs, pens, pencils and human skin.

3) Always wear gloves when handling kit components. Do not pick up any kit component with an ungloved hand.
4) Keep all kit components tightly sealed when not in use. Keep all tubes containing RNA tightly sealed during the incubation steps.

NOTE: *We strongly recommend the addition of the LGC, Biosearch Techonologies™ RiboGuard™ RNase Inhibitor or other RNAse inhibitor to each reaction.*

Choice of primer for first-strand cDNA synthesis

First-strand cDNA synthesis can be primed using either an **oligo(dT) primer**, **random primers** or **gene-specific primers** (all primers to be provided by the user).

An **Oligo(dT) primer** is the most commonly used method for priming first-strand cDNA synthesis when using an eukaryotic RNA sample. Oligo(dT) primes cDNA synthesis only from the poly(A) tail present at the 3′ end of almost all eukaryotic mRNAs. Since poly(A) RNA constitutes just 1-5% of the RNA in a eukaryotic total cellular RNA preparation, the complexity of the cDNA produced is significantly less than when the cDNA is synthesised using random primers. Lower-complexity cDNA can result in a more sensitive and specific PCR amplification. Additionally, priming cDNA synthesis with an oligo(dT) primer precludes the need to enrich the RNA sample for poly(A) RNA. Typically, the oligo(dT) primer is 18-21 nucleotides in length.

Random primers initiate cDNA synthesis from all RNA species (rRNA and mRNA) in a total cellular RNA sample. Since rRNA, which constitutes >95% of the RNA in a total RNA sample, is converted to cDNA using random primers, the complexity of the cDNA will be much greater than when priming the reaction with oligo(dT). As a result, random primers are much less frequently used than oligo(dT) primers. Random primers are typically 6-9 nucleotides in length and can be helpful when:

1) Synthesising cDNA from mRNAs that lack a poly(A) tail (such as bacterial mRNA) or have a very short poly(A) tail.
2) Priming cDNA synthesis from partially degraded RNA samples such as those obtained from formalin-fixed paraffin-embedded (FFPE) tissue samples.
3) Priming cDNA synthesis of a poly(A)-enriched RNA sample.
4) It is necessary to eliminate or reduce 3′ sequence bias that can result when using an oligo(dT) primer.

Gene-specific primers, designed and synthesised by the user, provide the greatest specificity when priming cDNA synthesis of an mRNA. However, the user frequently must determine the optimal primer annealling and extension (reverse transcription) conditions empirically for each primer used.

5. MMLV High Performance Reverse Transcriptase cDNA synthesis procedure

The following protocol has been optimised to convert 100 pg of cellular RNA to 1 µg of first-strand cDNA using an oligo(dT) primer or random primers. The use of gene-specific primers may require additional optimisation of the reaction.

Gently mix and briefly centrifuge all kit components prior to dispensing.

1. Denature the RNA sample and anneal the primer(s). For each reaction, combine the following components on ice, in a sterile (RNase-free) 0.2 mL or 0.5 mL tube:

x µL	RNase-free water
x µL	Total RNA sample (up to 1 µg)
10 pmol	Oligo(dT)$_{18-21}$ primer
	- or -
10 ng	Random primers
	- or -
x µL	Gene-specific primers
10 µL	Total reaction volume

2. Incubate at 65 °C for 2 minutes in a water bath or thermal cycler with heated lid.
3. Chill on ice for 1 minute. Centrifuge briefly in a microcentrifuge.
4. For each reaction, combine the following components on ice:

x µL	RNase-free water	
10 µL	Annealled RNA:primer(s)	(from step 3 above)
2 µL	MMLV HP RT 10X Reaction Buffer	
2 µL	100 mM DTT	
0.5 µL	RiboGuard RNase Inhibitor	(optional, sold separately)
1 µL	10 mM dATP Solution	(provided by the user, sold separately)
1 µL	10 mM dCTP Solution	(provided by the user, sold separately)
1 µL	10 mM dGTP Solution	(provided by the user, sold separately)
1 µL	10 mM dTTP Solution	(provided by the user, sold separately)
0.5 µL	MMLV High Performance Reverse Transcriptase (200 units/µL)	
20 µL	Total reaction volume	

5. Mix the reaction gently.
   If using oligo(dT) primers, incubate the reaction at 37 °C for 60 minutes.
   If using random primers, incubate the reaction at room temperature for 10 minutes and then at 37 °C for 60 minutes.
6. Terminate the reaction by heating at 85 °C for 5 minutes.
7. Chill on ice for at least 1 minute. Centrifuge briefly in a microcentrifuge.
8. The cDNA can be used immediately, without purification, for end-point or real-time PCR (qPCR), converted to double-stranded cDNA or stored at -20 °C for future use.

6. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.

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