Biomatik
Tel: (519) 489-7195, (800) 836-8089
Fax: (519) 231-0140, (877) 221-3515
Email: info@biomatik.com
http://www.biomatik.com

Product Information

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K5147 - M-MuLV First Strand cDNA Synthesis Kit

Components	K5147-100RXN
M-MuLV Reverse Transcriptase (200 U/µl)	100 µl
Oligo(dT) (10 µM)	100 µl
Random Primers (10 µM)	100 µl
dNTPs (10 mM)	100 µl
5X RT buffer	400 µl
Nuclease-free H2O	2 x 1.0 ml

Description

M-MuLV Reverse Transcriptase is a new generation reverse transcriptase, the best of its kind on the market. The enzyme catalyzes the synthesis of complementary DNA strands from single-stranded RNA or DNA templates. Due to a series of mutations introduced within the RNase H domain of this enzyme, there is no detectable RNase H activity associated with the enzyme. The lack of RNase H activity helps to eliminate RNA degradation during first strand cDNA synthesis, resulting in better yield and length of cDNA synthesized. Furthermore, M-MuLV Reverse Transcriptase contains an additional fidelity-enhancing subunit which drastically enhances accuracy in reverse transcription.

M-MuLV First Strand cDNA Synthesis Kit contains all components required for first strand cDNA synthesis, with the choice of using either Oligo(dT) and/or Random Primers. The Oligo(dT) anneals selectively to the Poly(A) tail of mRNAs. Random Primers do not require the presence of poly (A) and can be used for the transcription of mRNA 5'-end regions. Gene-specific primers may also be used with the kit. The first strand cDNA can be directly used as a template in PCR.

Unit Definition

One unit is defined as the amount of enzyme required to incorporate 1 nmol of deoxynucleotide into acid-precipitable material in 10 minutes at 37°C using Poly (A) and Oligo(dT) as template and primer, respectively.

Primer Selection

Oligo(dT) are oligonucleotides that anneal to the 3'-Poly(A) + mRNA. Therefore, only mRNA or total RNA templates with 3'-Poly(A) tails are used in cDNA synthesis.
Random Primers are oligonucleotides that anneal at non-specific sites of RNA templates. Therefore, all forms of RNA can be used in cDNA synthesis.
Gene-Specific Primers are oligonucleotides that are designed to anneal to the specific site of a target gene.

Storage Buffer

20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01 % (v/v) NP-40, 50 % (v/v) glycerol.

Storage Condition

Store all components at -20°C in a non-frost-free freezer. All components are stable for 1 year from the date of shipping when stored and handled properly.

Protocol

Reverse transcription reactions should be assembled in an RNase-free environment. The use of “clean”, automatic pipettes designated for PCR and aerosol-resistant barrier tips are recommended.

1. Thaw RNA templates and all reagents on ice.
2. Prepare the following reaction mixture on ice.

Components	Volume
5X RT Buffer	4 µl
dNTP (10 mM)	1 µl
Primers	1 µl
Total RNA or poly(A) + mRNA	Variable (1 ng – 2 µg/rxn)
M-MuLV Reverse Transcriptase	1 µl
Nuclease-free H2O	Up to 20 µl

3. Gently mix the reaction and briefly centrifuge.
4. Perform cDNA synthesis by incubating for 15 minutes at 50-55°C.
5. Stop the reaction by heating at 85°C for 5 minutes. Chill on ice. The newly synthesized first-strand cDNA is ready for immediate downstream applications, or for long-term storage at -20°C.

General Notes

1. Both poly(A) + mRNA and total RNA can be used for first strand cDNA synthesis, but poly(A) + mRNA may give higher yields and improved purity of final products.
2. RNA samples must be free of genomic DNA contamination.
3. Unlike Oligo(dT) priming, which requires little optimization, the ratio of Random Primers to RNA is often critical in terms of the average length of cDNA synthesized. A higher ratio of Random Primers to RNA will result in a higher yield of shorter (~500 bp) cDNA, whereas a lower ratio will lead to longer cDNA products.
4. To remove RNA complementary to the cDNA, add 1 µl (2 U) of E. coli RNase H and incubate at 37°C for 20 mins.

**** The kit is designed for laboratory research purpose only. Not for human or animal diagnostic and therapeutic use.***