Limited liability company
«Biolabmix»
TIN 5408278957 CAT 540801001
630090, Novosibirsk obl., Novosibirsk,
st. Injenernaya, building № 28
Tel/Fax: +7(383)363-51-91, Tel: +7(383)363-22-40
E-mail: sales@biolabmix.ru

M-MuLV –RH First Strand cDNA Synthesis Kit

Cat. Number: R01-50, R01-250

Product description

M-MuLV –RH First Strand cDNA Synthesis Kit is a complete system for efficient synthesis of first strand cDNA from mRNA or total RNA templates. The kit contains all necessary components for performing reverse transcription: MuLV –RH reverse transcriptase, 5× RT buffer mix, solutions of oligo(dT)$_{16}$ primer and random hexaprimer, and RNase-free water.

M-MuLV–RH enzyme is a genetically modified reverse transcriptase (revertase) from murine leukemia virus (M-MuLV) that is different from the wild-type M-MuLV in structure, catalytic features and temperature optimum of activity. The enzyme possesses RNA- and DNA-dependent polymerase activity but lacks RNase H activity. Temperature optimum for M-MuLV-RH activity is 42 °C (the enzyme remains active at temperatures up to 50 °C). The enzyme is able to synthesize first strand cDNA up to 7 kb and incorporate modified bases.

The kit includes two types of buffers based on KCl and $(NH_4)_2SO_4$, optimized for theeffective reverse transcription reaction from all of the RNA matrixes, including complicated segments (highly structured or GC-rich).

Oligo(dT)$_{16}$ primer and random hexaprimer included in the kit allow more targeted approach for the study of any RNA type or region by reverse transcription. Random hexaprimer non-specifically binds to RNA template and is used for cDNA synthesis from any RNA type contained in total RNA. Oligo(dT)$_{16}$ primer selectively anneals to the 3'-poly(A)-termini of RNA enabling cDNA synthesis only from mRNA containing a poly(A)-end. Gene-specific primer can be also used for the synthesis of specific sequence.

Product composition:

Component	Cat. № (amount)	
	R01-50 (50 reactions)	R01-250 (250 reactions)
M-MuLV–RH reverse transcriptase, 100 u/µl*	1 × 50 µl (5000 U)	1 × 250 µl (25000 U)
5× RT buffer (KCl)	1 × 220 µl	3 × 400 ml
5× RT buffer ($(NH_4)_2SO_4$)	1 × 220 µl	3 × 400 ml
20× dNTP mix (10 мМ each)	1 × 120 µl	2 × 300 ml
Dithiothreitol, 0.1 M	1 × 110 µl	2 × 260 ml
Random hexaprimer, 20 µM	1 × 50 µl	1 × 260 µl
Oligo(dT) primer, 20 µM	1 × 50 µl	1 × 260 µl

* One activity unit is the amount of enzyme required to catalyze incorporation of 1 nmol of dNTP into an acid-insoluble product in 10 min at 37 °C.

Applications:

- First strand cDNA synthesis for RT-PCR and qRT-PCR
- cDNA synthesis for cloning
- Synthesis of labeled cDNA probes for microarrays
- DNA labeling
- RNA analysis by primer extension

M-MuLV–RH reverse transcriptase features:

- Synthesis of complementary DNA strand from RNA template (RNA-dependent DNA polymerase)
- Lacks RNase H activity
- Allows to obtain long cDNA fragments up to 7 kb
- Provides high yield of cDNA: the use of 100 U of enzyme per 1 µg of RNA provides not less than 100 ng of first strand cDNA
- Improved thermostability
- Contains RNase inhibitor

Source

The enzyme is obtained from recombinant strain of *E. coli* expressing deletion variant of M-MuLV revertase gene.

Storage buffer:

50 mM Tris-HCl (pH 8.0 at 25 °C), 100 mM NaCl, 1 mM EDTA, 5 mM dithiothreitol, 50 % (v/v) glycerin, and 0.1 % (v/v) NP-40.

5× RT buffer (KCl):

250 mM Tris-HCl, pH 8.3 (at 25 °C), 250 mM KCl, 20 mM $MgCl_2$, stabilizers.

5× RT buffer ($(NH_4)_2SO_4$):

250 mM Tris-HCl, pH 8.5 (at 25 °C), 100 mM $(NH_4)_2SO_4$, 20 mM $MgCl_2$, stabilizers.

Protocol:

Before starting to work, we highly recommend to study the rules and guidelines presented in the description of the kit on site: http://biolabmix.ru/catalog

Reverse transcription – polymerase chain reaction (RT-PCR)

I. Reverse transcription (first strand cDNA synthesis)

Defrost kit components, vortex the solutions and collect all droplets from tube walls by brief centrifuging. Keep the tubes on ice during work.

1. Add the following components in sterile, nuclease-free tube on ice in the following order:

RNA template	total RNA or poly(A) mRNA or specific RNA	0.1 pg – 5 µg 10 pg – 0.5 µg 0.01 pg – 0.5 µg
Primer	oligo(dT)$_{16}$ or random hexaprimer or gene-specific	1 – 3 µl 1 – 3 µl 15-20 pmol
DEPC-treated water		up to 12 µl
	Total volume	12 µl

2. Carefully vortex and collect droplets by centrifuging. Heat mixture at 70 °C for 2-3 min in order to melt secondary structures and place the tube on ice.

Note: this procedure is mostly required when using a random hexaprimer and/or highly structured or GC-rich templates.

3. Add a premix of the following composition:

5× RT buffer mix	4 µl
0.1 M DTT	2 µl
10 mM dNTPs mix	1 µl
M-MuLV–RH revertase (100 u/µl)	1 µl
Total volume	8 µl

4. Carefully vortex and collect drops by centrifuging.

5. When using oligo(dT)$_{16}$ or gene-specific primer for cDNA synthesis, incubate the reaction solution at 42 °C for 60 min. If using random hexaprimer, incubate at 25 °C for 10 min and then at 42 °C for 60 min.

Note: if RNA template is GC-rich or highly structured, the reaction can be performed at higher temperatures (45-50 °C).

6. The reaction is stopped by heating of the reaction solution at 70° C for 10 min.

The product of reverse transcription reaction can be used directly for PCR amplification or stored at -20 °C for at least one week. For long-term storage -70 °C temperature is recommended.

II. PCR amplification of first strand cDNA

The product of first strand cDNA synthesis can be used directly for conventional PCR or real-time PCR. The required volume of the reaction solution after performed reverse transcription is not more than 1/10 of the total volume of PCR reaction mixture. Usually, 2 µl of RT reaction solution is used as a template for further PCR in 50 µl volume. For standard amplification (conventional PCR) of a fragment up to 5 kb the following kits can be used: **BioMaster HS-Taq PCR-Color (2×)** (MHC10-200, MHC10-1020), **BioMaster HS-Taq PCR (2×)** (MH10-200, MH10-1020). **BioMaster LR HS-Taq PCR-Color (2×)** (MC040-40, MC040-200) or **BioMaster LR HS-Taq PCR (2×)** (M040-40, M040-200) are recommended for fragments of more than 5 kb in length. For real-time PCR amplification we recommend kits **BioMaster HS-qPCR Mix (2×)** (MH020-200, MH020-1020) and **BioMaster qPCR Mix SYBR (2×)** (MH030-200, MH030-1020).

Optimization of reaction conditions:

1. If necessary, the reaction volume van be varied from 10 to 50 µl, while the amounts of all components should be changed proportionally.
2. The shorter the fragment of cDNA is the smaller amount of enzyme should be, added per reaction.

The recommended amount of M-MuLV–RH revertase per reaction of 20 µl volume:

Length of synthesized cDNA	The amount of RNA		
	< 500 ng	< 500 ng	> 2 µg
50 – 600 bp	10 – 25 U	50 – 600 bp	10 – 25 U
600 – 2000 bp	25 – 100 U	600 – 2000 bp	25 – 100 U
> 2000 bp	50 - 100 U	> 2000 bp	50 - 100 U

3. Increasing the concentration of RNA template in the reaction solution leads to the increase in total reaction yield.

Note: if the amount of RNA template in the reaction mixture is more than 2 µg per 20 µl of reaction solution, it is recommended to increase not only the concentration of M-MuLV-RH revertase but primer concentration 1.5 - 2 times as well in order to increase the reaction yield.

4. In order to facilitate the reverse transcription of template areas containing GC-rich regions or regions with complicated secondary structure, it is recommended to use random hexaprimer (Random $(dN)_6$).

Note: in case of using complex templates, the temperature can be increased up to 45-47 °C (temperature increase up to 50 °C can result in decreased reaction yield).

Storage conditions: at -20 ° C - 12 months; not more than 30 thawing-freezing cycles.

Transportation: Transport in thermocontainers with cooling elements; the ambient temperature increment to the room temperature during the transportation up to 7 days is allowed.