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

Reverse transcriptase M-MuLV–RH

Cat. No. R03-10, R03-50

Product description

M-MuLV –RH reverse transcriptase (revertase) is a genetically modified enzyme from murine leukemia virus (M-MuLV). M-MuLV –RH 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 is 42°C (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.

In addition to M-MuLV –RH enzyme, the kit includes 5× RT buffer mix containing all components necessary for reverse transcriptase performance, except for primers and RNA template. The buffer is optimized for efficient reverse transcription of a wide range of RNA templates.

Product composition

Component	Cat. # (amount)	
	R03-10	R03-50
M-MuLV –RH Reverse Transcriptase, 100 u/µl*	1 × 100 µl (10000 u)	2 × 250 µl (50000 u)
5× RT buffer mix	1 × 1 ml	4 × 1.25 ml
Primers-mix	1 × 200 µl	2 × 500 µl

* One activity unit (u) is the amount of enzyme that incorporates 1 nmol of dTMP into an acid-insoluble product in 10 min at 37 °C.

Area of application

- First strand cDNA synthesis for RT-PCR and real-time RT-PCR
- cDNA generation for cloning
- Synthesis of labeled cDNA probes for microarrays
- DNA labeling

Features of M-MuLV –RH reverse transcriptase

- Synthesis of the 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 units of enzyme per 1 µg of RNA provides not less than 100 ng of first strand cDNA
- Improved thermostability
- Contains RNAse inhibitor

Storage buffer:
50 mM Tris-HCl (pH 8 at 25 °C), 100 mM NaCl, 1 mM EDTA, 5 mM dithiothreitol, 50 % (v/v) glycerol, and 0.1 % (v/v) NP-40.

5× RT buffer mix:
250 mM Tris-HCl (pH 8.3 at 25 °C), 250 mM KCl, 20 mM $MgCl_2$, 2.5 мМ each deoxynucleoside triphosphate, 50 mM dithiothreitol, stabilizers and enhancers.

Protocol
Before starting to work, we recommend to study the guidelines and recommendations given in the kit description on our site http://biolabmix.ru

Reverse transcription – polymerase chain reaction (RT-PCR)

1) Reverse transcription (synthesis of first strand cDNA)
After defrosting kit components, stir the mixtures and remove droplets form the tube walls using microcentrifuge. Keep the tubes on ice during work.
Note: If precipitation is observed in the 5× OT-buffer-mix, heat the solution to 45-50 °C and stir until dissolved. Do not allow the buffer to incubate at room temperature or above for long periods of time unnecessarily. This may lead to a decrease in DTT concentration and a drop in reaction efficiency.

1. Add the following reagents into sterile, nuclease-free tubes on ice as listed below:

RNA template	Total RNA or poly(A) mRNA or specific RNA	0.1 ng – 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. Mix carefully and spin down the droplets by centrifugation.

Heat the solution for 2-3 minutes at 70 ° C in order to melt secondary structures and place the tube on ice.

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

3. Добавить предварительно приготовленную смесь следующего состава:

5× RT buffer mix	4 µl
M-MuLV –RH revertase (100 u/µl)	1 µl
DEPC-treated water	3 µl
Total volume	8 l

4. Vortex carefully and put down the drops by centrifugation.
5. In case of using an oligo(dT)$_{16}$ or a gene-specific primer for cDNA synthesis, incubate

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

the reaction solution for 60 min at 42 °C. In case of using a random hexaprimer, incubate the solution at 25 °C for 10 min and then at 42 °C for 60 min.

6. To stop the reaction, heat the solution at 70 °C for 10 min. The product of reverse transcription can be directly used for PCR amplification or stored at -20 °C for a week or even more. For long-term storage, keep the product at -70 °C

2) PCR amplification of first strand cDNA

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

Optimization of reaction conditions

1. If necessary, the reaction volume can be varied from 10 to 50 µl with proportional change in the amount of all components.
2. The shorter a cDNA fragment is, the smaller amount of enzyme is required per reaction.

Recommended amount of M-MuLV –RH revertase per 20 µl reaction:

Length of synthesized cDNA	Amount of RNA template		
	< 500 ng	500 ng – 2 µg	> 2 µg
50 – 600 kb	10 – 25 u	25 – 50 u	50 – 100 u
600 – 2000 kb	25 – 100 u	50 – 100 u	200 – 300 u
> 2000 kb	50 -100 u	100 u	200 – 300 u

Increased concentration of RNA template in the reaction solution leads to elevated reaction yield.

Note: in case if the amount of RNA template in the reaction solution is more than 2 µg per 20 µl reaction, it is highly recommended to increase primer concentration 1.5 to 2 fold alongside with M-MuLV –RH revertase concentration in order to increase the reaction yield.

3. To facilitate transcription of template regions containing GC-rich areas or areas of complicated secondary structure, it is highly recommended to use random hexaprimer (Random $(dN)_6$).

Note: in case of using complicated templates, the reaction temperature can be elevated up to 45-47 °C (elevation of temperature up to 50 °C results in lower reaction yield, but provides better transcription of structured areas).

Storage conditions: at -20 ° C - 1 year; not more than 30 thawing-freezing cycles.

Transportation: at 0 - +4 ° C.