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 RNAscribe RT

Cat. Number: R04-10, R04-50

Description:

RNAscribe RT – genetically modified reverse transcriptase (revertase) from murine leukemia virus (M-MuLV). It differs from wild type M-MuLV by structure, catalytical properties and activity temperature optimum. The enzyme shows RNA- and DNA-dependent polymerase activity and has the optimal activity at 55 °C (active up to 65 °C). The enzyme has a capacity to synthetize first cDNA chain with the length up to 9 t.b. and include modified bases. Its fast reaction velocity allows to perform synthesis in just 15 minutes, and the enzyme high working temperature (up to 65 °C) allows to use complicated matrixes and provides reaction specificity.

The kit also includes 5 × RT-buffer-mix, which contains all of the necessary componentsfor revertase functioning, excluding primera and RNA-matrix. The buffer contains is optimized for effective reverse transcription reaction performance from wide range of RNA-matrixes.

The kit is set to provide flexibility while preparing the reaction and allows to perform, RNA pre-incubation with primers.

Kit contains:

Component	Cat. № (Amount)	
	R04-10	R04-50
RNAscribe RT revertase, 100 U. A./µl*	1 × 100 µl (10000 U.A.)	2 × 250 µl (50000 U.A.)
5× RT-buffer-mix	1 × 1 ml	4 × 1,25 ml
Primer-Mix	1 × 0,2 ml	2 × 0,5 ml

* The activity unit is accepted as an amount of the enzyme, catalyzing the inclusion of 1 nmoles dTMP in acid-soluble product in 10 min at 37 °C.

Application

- Synthesis of the first cDNA chain for RT-PCR and real-time RT-PCR;
- Synthesis of cDNA for cloning;
- Synthesis of cDNA from long and complicated matrixes;
- Labeled cDNA probes production for microarrays;
- DNA labeling.

RNAscribe RT revertase properrties

- Performes synthesis of complimentary DNA chain on RNA-matrix (RNA dependent DNA polymerase);
- Inhibited byRNAse H activity;
- Allows to synthetize fragments with the length up to 9 t.b.;

- Provides high cDNA yield: using 100 U.A. of enzyme with 1 µg RNA reaction yield is no less than 100 ng of first cDNA chain;
- Has increased thermostability;
- Contains RNAse inhibitor.

Source
The enzyme was obtained from recombinant *E. coli* strain, containing the plasmid with overexpressing revertase RNAscribe RT gene.

Containment 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-mix:
250 mM Tris-HCl, pH 8.3 (at 25 °C), 250 mM KCl, 20 mM $MgCl_2$, 2.5 mM of each deoxynucleosidetriphosphate, 50 mM dithiothreitol, stabilizers and enchansers.

Assay
Before the start of the work it is recommended to read the rules and guidelines, listed in the kit description at http://biolabmix.ru

Reverse transcription – polymerase chain reaction (RT-PCR)

I. Reverse transcription (first cDNA chain synthesis)

After thawing of the kit components, vortex and discard the droplets on the tube walls and cap, using microcentifuge. During the workflow keep the tubes on ice.
Note: if precipitation in 5× RT-buffer-mix is observed, heat the solution up to 45-50 °C and vortex until precipitate dissolution.

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

RNA matrix	Total RNA Or poly(A) mRNA Or specific RNA	0.1 ng – 5 µl 10 pg – 0.5 µg 0.01 pg – 0.5 µg
Primers	Primer-mix Or gene-specific	1 – 3 µl 15-20 pmol
DEPC treated water	Up to 12 µl	
	Total volume	12 µl

2. Carefully mix and discard the droplets by centrifuging.

Heat the mixture for 2-3 min at 70 °C for the secondary structure melting and put the tube on ice.
Note: this procedure is not necessary, but preferable, is using the random hexaprimer and\or highly structured or GC-rich matrixes.

3. Add the pre-made mix with the next contains:

5× RT-buffer-mix	4 µl
RNAscribe RT revertase (100 U./µl)	1 µl
DEPC treated water	3 µl
Total volume	8 µl

4. Carefully mix and discard the droplets by centrifuging.
5. While using oligo(dT)$_{16}$ or gene-specific primer for cDNA synthesis, incubate the reaction mixture for 30-50 min at 55 °C. In case of random hexaprimer or Primer-Mix application, it is recommended to add incubation for 10 min at 25 °C and after 30-50 min at 55 °C.

Note: if RNA matrix is GC-rich or structured, reaction may be conucted at the higher temperature (up to 65 °C).

6. To stop the reaction, the mixture heats up to 85 °C for 5 min.

Reverse transcription reaction product can be used directly in PCR-amplification or stored at -20 °C at least for one week. For the longer storage temperature -70 °C is recommended.

II. PCR-amplification of the first cDNA chain

The first cDNA chain synthesis product can be used directly in standrt PCR or real-time PCR. The required volume of the reaction mixture after the reverse transcription is less than 1/10 from the total PCR reaction mixture volume. Normally, 2 µl of RT reaction mixture is used as a matrix for the subsequent PCR in 50 µl volume. For the amplification of the fragment up to 5 t.b. in standart PCR **BioMaster HS-Taq PCR - Color (2×)** (MHC10-200, MHC10-1020) and **BioMaster HS-Taq ПЦР (2×)** (MH10-200, MH10-1020) can be used. For the fragments longer than 5 t. b. it is recommended to use **BioMaster LR HS-Taq PCR-Color (2×)** (MHC040-100, MHC040-400) or **БиоМастер LR HS-Taq ПЦР (2×)** (MH040-100, MH040-400). For real-time PCR amplification we recommend to use kits **BioMaster HS-qPCR (2×)** (MH020-400, MH020-2040), **BioMaster HS-qPCR SYBR Blue (2×)** (MHC030-400, MHC030-2040).

Reaction condition optimisation

1. If needed, reaction volume may be varied from 10 to 50 µl, proportionally changing amount of all the components.
2. The shorter the cDNA fragment, the lesser amount of the enzyme should be added to the reaction.

Recommended amount of RNAscribe RT revertase per 20 µl reaction volume:

The length of synthetized cDNA	RNA matrix amount	
	< 1 ng	500 ng – 2 µg
100 – 2000 b.p.	25 – 100 U. A.	75 – 150 U. A.
More than 2000 b.p.	100 -150 U. A.	100 - 250 U. A.

Increment of RNA matrix concentration in the reaction mixture leads the increment of the total reaction yield

Note: if the amount of RNA matrix in the reaction mixture exceeds 2 µg per 20 µl reaction, to increase the reaction yield it is recommended to bot only increase the M-MuLV–RH revertase concentration, but also the concentration of primers by 1,5 - 2 times.

3. To facilate the passing through the matrix GC-rich or complicatedly structured, random hexaprimer (Random $(dN)_6$) is recommended to apply.

Note: in case of complex matrixes temperature can be increased to 55-60 °C (the temperature increment up to 65 °C will lead to the decrement of the reaction yield, but contributes to passing through the structured regions).

Storage: at -20°C – 1 year; max. of 30 freeze-thaw cycles. The enzyme is resistant to room-temperature incubation (up to 7 days).

Transportation: in thermocontainers with cooling elements; the temperature may increase up to the ambient temperature if transportation lasts less than 7 days.