Product information

Product Name: ExcScript Reverse Transcriptase

Catalog No: VN50RT, VN51RT

Packing Size: 1 x 50 µl and 4 x 50 µl

Shipping Condition: Dry ice / Ice pack

Storage Condition: -20^{0}C

Product Description:

ExcScript Reverse Transcriptase (RT) was developed through in vitro evolution of M-MuLV RT. The enzyme possesses RNA-dependent and DNA-dependent polymerase activity but lacks RNase H activity, which is due to a mutation in the RNase H domain of M-MuLV RT. Compared to wild type M-MuLV RT, the engineered enzyme has dramatically improved thermostability, inhibition resistance, 50-fold higher processivity, robustness, and increased synthesis rate.

The lack of RNase H activity allows ExcScript to produce long RNA transcripts of up to 20 kb. Additionally, the enzyme's high thermostability enables it to maintain full activity during the entire reverse transcription reaction and generate high yields of cDNA. The reaction temperature can be increased up to 65°C to efficiently transcribe RNA regions with high secondary structures or to improve specificity using gene-specific primers. The high processivity of ExcScript results in increased resistance to common reaction inhibitors.

Product Features

- Thermostable—90% active after incubation at 50°C for 60 minutes in a reaction mixture
- Active up to 65°C
- High yields of full-length cDNA up to 20 kb
- High sensitivity—reproducible cDNA synthesis from a wide range of starting total RNA amounts (1 pg to 5 µg)
- Efficient—complete cDNA synthesis in 15 to 30 minutes
- Increased resistance to common reaction inhibitors
- Incorporates modified nucleotides

Applications

- First strand cDNA synthesis for RT-PCR and real-time RT-PCR.
- Reverse transcription at elevated temperatures to reduce effects of secondary structure.
- Synthesis of cDNA for cloning and expression.
- Generation of labeled cDNA probes for microarrays.
- Analysis of RNA by primer extension.

Protocol for First Strand cDNA Synthesis

The following is a general protocol for first-strand cDNA synthesis:

Mix and briefly centrifuge all reagents after thawing, keep on ice.

1. Add reaction components into a sterile, nuclease-free tube on ice in the indicated order:

Template RNA	Total RNA	1 pg-5 ug
	Poly(A) RNA	0.1 pg-500 ng
	Specific RNA	0.01 pg-500 ng
Primer	Oligo(dT)$_{18}$	1 µl (100 pmol)
	Random Hexamer	1 µl (100 pmol)
	Gene-specific Primer	15-20 pmol
dNTP Mix, 10 mM each		1 µl (0.5 mM final concentration)
Water, nuclease-free		To 14.5 ul

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2. Optional: If the RNA template is GC-rich or is known to contain secondary structures, mix gently, centrifuge briefly and incubate at 65°C for 5 min. Chill on ice, briefly centrifuge again and place on ice.
3. Add the following reaction components in the indicated order:

5X RT Buffer	4 ul
RNase Inhibitor	0.5 ul
ExcScript Reverse Transcriptase	50-200 U*
Total volume	20 ul

*To generate highest absolute amounts of RT reaction products (in applications such as synthesis of labelling probes) use 200 U of enzyme per reaction. For downstream applications, such as PCR or qPCR optimize enzyme amounts within a range of 50 U to 200 U.

Mix gently and centrifuge briefly.

4. Incubate:

- if a random hexamer primer is used, incubate for 10 min at 25°C followed by 30 min at 50°C.
- if an oligo(dT)$_{18}$ primer or gene-specific primer is used, incubate for 15-30 min at 50°C.
- For transcription of GC-rich RNA, the reaction temperature can be increased to 65°C.

5. Terminate the reaction by heating at 85°C for 5 minutes.

Note

- The reverse transcription reaction product can be used directly in PCR or qPCR, or stored at -20°C for up to one week. For longer storage, -70°C is recommended. Avoid freeze / thaw cycles of the cDNA.
- Use 2 µL of the cDNA reaction in 50 µL of PCR mix.

Recommendations for two-step RT-qPCR

- Priming: use a mix of oligo (dT)18 and random primers 25 pmol each per 20 µL reaction.
- Incubation: 10 min at 25°C followed by 15 min at 50°C.

Recommendations for long RT-PCR (>5 kb)

- Priming: oligo (dT)18 or gene specific primer should be used.
- Enzyme amount: use 20 U of ExcScript Reverse Transcriptase per reaction. 1X RT buffer can be used to dilute the enzyme just prior to reaction.
- Incubation: 30 min at 50°C.

Note: This product is for R&D use only

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