enzynomics

TOPscript™ III Reverse Transcriptase (High fidelity)

Cat.#	Size	Conc.
RT004S	10,000 units	200 units/μl
RT004M	20,000 units	200 units/μl
RT004L	50,000 units	200 units/μl
RT004H	50,000 units	1000 units/μl

Store at -20℃

Supplied with: 10X TOPscript™ RT Buffer
dNTP Mixture (2 mM each)
Sterile water (RNase free)

India Contact:

Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura,
Delhi – 110034 (INDIA).
Ph: +91-11-42208000, 42208111, 42208222
Mobile: +91-9810521400
Fax: +91-11-42208444
Email: customerservice@lifetechindia.com
Web: www.lifetechindia.com

Product description

TOPscript™ III Reverse Transcriptase (High fidelity) is a blend of a recombinant reverse transcriptase and a proofreading enzyme. The synergistic effect of both enzymes generate 7-fold higher fidelity compared to other commonly used reverse transcriptases. Due to the higher thermostability of reverse transcriptase and the specially optimized buffer system, reverse transcription reaction is possible at temperatures up to +55℃. TOPscript™ III Reverse Transcriptase (High fidelity) does not degrade RNA strands from RNA-DNA hybrids and therefore is capable of high yield of synthesizing cDNA. The optimum temperature of TOPscript™ III Reverse Transcriptase (High fidelity) is 50℃. TOPscript™ III Reverse Transcriptase (High fidelity) does not show inhibitory effect on one-step RT-PCR reaction.

Caracteristics

- Themostable and RNase H variant of M-MLV RTase
- Synthesize cDNA at 42℃~55℃.
- Especially the highest activity at 50℃
- Generate full-length transcripts up to 14 kb with the oligo(dT) primers.
- Increase accuracy during reverse transcription reactions.
- Efficiently synthesize cDNA by inhibiting the formation of secondary structure of RNA.

For Research Use Only. Not for use in diagnostic procedures.

ISO9001 ISO14001 ISO13485

Applications

- Cloning genes of interest
- Sequencing transcriptomes
- Generating cDNA libraries with large and full-length inserts
- Generating gene expression analysis, like RNA splicing analysis.

Unit definition

One unit is the amount of enzyme required to incorporate 1 nmol dTTP into acidinsoluble materials using 0.4 mM poly(rA)-oligo(dT) as substrate at 37℃ in 10 min.

Storage buffer

20 mM Tris-Cl (pH 7.5), 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.01% NP40, 50% glycerol

10X TOPscript™ RT Buffer

500 mM Tris-HCl (pH 8.3), 30 mM MgCl, 100 mM DTT, 750 mM KCl

Note

TOPscript™ Reverse Transcriptase performs optionally over the full range of 42℃-60℃. Typically, 50℃ is a good starting point. For RNAs containing secondary structure and other challenging targets, a synthesis temperature of 60℃ may be used without loss of performance.

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Standard reaction conditions

10X TOPscript™ RT Buffer	2 μl
TOPscript™ III Reverse Transcriptase (200 units/μl)	1 μl
dNTP mixture (2 mM each)	2 μl
[a]Template RNA	X μl
[b]Primer	1 μl
RNase Inhibitor (40 units/μl)	0.5 μl
Sterile water (RNase free)	up to 20 μl

[a]Prepare one of the following RNA template.
- Total RNA: 1 ng~5 μg
- Messenger RNA (mRNA): 1 ng~250 ng
- Specific RNA: 0.01 pg~0.5 μg

[b]Prepare one of the following primers.
- Oligo $(dT)_{18}$: 50 μM~100 μM
- Random hexamer: 50 μM~100 μM
- Specific primer: 15 pmol~20 pmol

→An additional annealing step is recommended:
· if using oligo$(dT)_{18}$, incubate at 42℃ for 5 min.
· if using random hexamer, incubate at 25℃ for 10 min.
→Incubate at 42℃-60℃ for 60 min.
→Incubate at 95℃ for 5 min to inactivate the reaction.

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