enzynomics

TOPscript™ II Reverse Transcriptase (RNase H-)

Cat.#	Size	Conc.
RT003S	10,000 units	200 units/μl
RT003M	20,000 units	200 units/μl
RT003L	50,000 units	200 units/μl
RT003H	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.
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Product description

TOPscript™ II Reverse Transcriptase (*RNase H-*) is genetically engineered recombinant protein that possesses moderate thermostability (42~55℃) and lacks RNase H activity. TOPscript™ II Reverse Transcriptase (*RNase H-*) does not degrade RNA strands from RNA-DNA hybrids and therefore is capable of high yield of synthesizing cDNA. The optimum temperature of TOPscript™ II Reverse Transcriptase (*RNase H-*) is 50℃. TOPscript™ II Reverse Transcriptase (*RNase H-*) does not show inhibitory effect on one-step RT-PCR reaction.

Characteristics

- Themostable and RNase H variant of M-MLV RTase (*RTase H-*)
- Synthesize cDNA at 42~55 ℃
- Especially the highest activity at 50 ℃
- Synthesis of long length cDNA (< 14 kb)
- Efficiently synthesize cDNA by inhibiting the formation of secondary structure of RNA

Applications

- First-strand cDNA synthesis
- RT-PCR
- Real-Time PCR
- Primer extension analysis

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

ISO9001 ISO14001 ISO13485

Quality control

- Purity: >99% on SDS-PAGE
- Endonuclease-free
- Exonuclease-free
- RNase-free
- Inhibitor-free
- Yield and length of cDNA product

Unit definition

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

Storage buffer

20 mM Tris-Cl (pH7.5), 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 $MgCl_2$, 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™ II 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 $(dT)_{18}$: 50~100 μM
- Random hexamer: 50~100 μM
- Specific primer: 15~20 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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