enzynomics

TOPscript™ Reverse Transcriptase

Cat.#	Size	Conc.
RT002S	10,000 units	200 units/μl
RT002M	20,000 units	200 units/μl
RT002L	50,000 units	200 units/μl
RT002H	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,
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Fax: +91-11-42208444
Email: customerservice@lifetechindia.com
Web: www.lifetechindia.com

Product description

TOPscript™ Reverse Transcriptase is genetically engineered version of M-MLV RT which is highly thermostable, thus can synthesize cDNA at elevated temperatures up to 60℃. This property is very useful when RNA templates are long and have extensive secondary structures. TOPscript™ Reverse Transcriptase is capable of synthesizing cDNA longer than 20 kb from messenger RNA.

Characteristics

- Molecular weight: 71 kDa
- Broad reaction temperature: 37℃~60℃
- Synthesis of long cDNA
- Excellent sensitivity

Applications

- Synthesis of first-strand cDNA,
- Array labeling
- cDNA library construction
- 3' and 5' RACE, RT-PCR
- Primer extension

Quality control

- Purity: >99% on SDS-PAGE
- Endonuclease-free
- Exonuclease-free
- RNase-free
- Inhibitor-free
- Satisfactory yield and length of cDNA products

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

ISO9001 ISO14001 ISO13485

Unit definition

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

Life Technologies™
Your Molecular & Cell Technology Partner

Standard reaction conditions

10X TOPscript™ RT Buffer	2 μl
TOPscript™ Reverse Transcriptase (200 units/μl)	1 μl
dNTP Mixture (2 mM each)	1 μl
a)Template RNA	X μl
b)Primer	1 μl
RNase Inhibitor (40 units/μl)	0.5 μl
Distilled water	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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