TOPOTAQ DNA POLYMERASE

Cat #	Size	Conc	Storage
T016	100 U	3.0 U/µl	+4 to +8°C (do not freeze)
T056	500 U	3.0 U/µl	
T256	2500 U	3.0 U/µl	

DESCRIPTION

TopoTaq is a hybrid DNA polymerase that offers exceptional performance for a wide spectrum of PCR applications. Incorporating a new technology developed by Fidelity Systems (1), TopoTaq dramatically advances the key properties of the *Taq* DNA polymerase by linking it with unique non-specific DNA binding domains. TopoTaq utilizes the gain in processivity, thermostability and specificity, resulting in shortened extension times, more robust and high yield amplification, and the ability to do 20 kb templates in the absence of exonuclease activities. The extra amplification performance of TopoTaq is further achieved by incorporating a hyperstable *Methanopyrus* DNA topoisomerase (2,3) that facilitates DNA strand separation. TopoTaq demonstrates consistent results on G+C-rich templates and can produce up to 12 kb long products from G+C-rich genomic DNA. It becomes possible due to the powerful strand displacement capability of TopoTaq and due to the unlinking activity of the *Methanopyrus* topoisomerase.

Sufficient reagents are provided for 100/500/2500 amplification reactions of 20 µl volume or 40/200/1000 reactions in 50 µl volume.

Component	Amount/Cat #		
	T016	T056	T256
TOPOTAQ DNA Polymerase	34 µl	170 µl	850 µl
2X TOPOTAQ Amplification Buffer with 6 mM $MgCl_2$*	1.0 ml	5.0 ml	25 ml
1X TOPOTAQ Dilution buffer	100 µl	510 µl	2.55 ml

* Dissolve precipitate (if any) at 37˚C before use

PCR PROTOCOL

The following general procedure is suggested as a guideline and as a starting point when using TOPOTAQ DNA polymerase in any PCR amplification reaction.

1. Add the following components to an autoclaved microcentrifuge tube on ice (preferably) or at ambient temperature:

Components	Volume µl		Final Conc
2X Amplification Buffer with 6 mM $MgCl_2$	10	25	1X; 3 mM $MgCl_2$ *
dNTP mixture (10 mM each dNTP)	1.0	2.5	0.5 mM each
Primer mixture (10 µM each)	0.6-1.0	1.5-2.5	0.3-0.5 µM each
DNA (1-100 ng)**	≥ 1	≥ 1	
TOPOTAQ (diluted 1:3 in 1X Dilution buffer)	1.0	2.0	1-2 U ***
Distilled water	to 20	to 50	

NOTE: *Make sure that the concentration of Mg^{2+} exceeds total concentration of dNTPs and other chelators (such as EDTA) by 1-1.5 mM.
** Use 10 pg – 10 ng of low complexity DNA (plasmid, phage) or 20-200 ng of high complexity DNA (mammalian).
*** For most targets 1 unit is sufficient If less than 1 pg plasmid DNA (1 ng genomic DNA) is used for amplification, dilution of enzyme 1:5 instead of 1:3 might be helpful. When amplifying targets above 5 kb more enzyme may be required.

2. Mix contents of the tubes and overlay with mineral or silicone oil, if necessary.

3. Cap the tubes and centrifuge briefly to collect the contents.
4. Denature the template for 1-2 min at 94-98°C. Perform 20-30 cycles of PCR amplification as follows:

Three-step cycling	Two-step cycling
Denature: 94-98°C, 5-30 s	Denature: 94-98°C, 5-30 s
Anneal: **50±5**°C, 30 s*	
Extend: **68**±2°C, 0.3-1 min/1kb	Extend 68±2°C, 0.3-1 min/1kb
Final Step: 72°C, 6 min	Final Step: 72°C, 6 min

NOTE: Two-step cycling can be used for long primers with high Tm. Denaturing time depends on the DNA template and on the thermocycler used.
*Reduce annealing temperature or use longer primers (≥22 nt) if no product occurs.

5. Maintain the reaction at 4°C after cycling. The samples can be stored at -20°C until use.
6. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide staining.

UNIT DEFINITION

One unit of TOPOTAQ DNA Polymerase determined by the initial rate of DNA synthesis with 1 µM fluorescent primer-template junction (PTJ) duplex substrate at 70^0C, in a standard primer extension assay (1), is equivalent to one unit of a DNA polymerase that will incorporate 10 nmoles of deoxyribonucloeotides into acid insoluble material.

LIMITED PRODUCT WARRANTY

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REFERENCES

1. Pavlov *et al.* (2002) Helix-hairpin-helix motifs confer salt resistance and processivity on chimeric DNA polymerases. *Proc. Natl. Acad. Sci. USA* **99**, 13510-13515
2. Journal Club (2002) A happy marriage: advancing DNA polymerases with DNA topoisomerase supplements *Trends in Biotech* **20**, 491
3. Pavlov *et al.* (2004) Thermostable chimeric DNA polymerases with high resistance to inhibitors. In: DNA Amplification: Current Technologies and Applications (eds. Demidov, V. & Broude, N.) Horizon Bioscience, pp 3-20.

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