TOPOTAQ HF (HIGH FIDELITY) DNA POLYMERASE

Cat #	Size	Conc	Storage
H016	100 U	3.0 U/µl	+4 to +8°C (do not freeze)
H056	500 U	3.0 U/µl	

DESCRIPTION

TOPOTAQ HF is a unique high fidelity DNA polymerase mixture containing hybrid TopoTaq DNA polymerase (1-3) and a hyperstable *Methanopyrus* DNA topoisomerase. The added non-specific DNA binding domains to the core Taq DNA polymerase domain, a 3'-5' proofreading domain from the *Methanopyrus* family B polymerase, and the DNA unlinking activity of the topoisomerase combine the strength of all components for a thermal cycling reaction, and provide unsurpassable performance and results. TOPOTAQ HF, owing to the presence of the proofreading domain and DNA topoisomerase, offers high specificity and ten times the fidelity of *Taq* DNA polymerase alone, while the proprietary non-specific DNA binding domains provide extreme processivity and ultra-fast chain extension capability of TOPOTAQ HF. TOPOTAQ HF amplifies highly G+C-rich targets (up to 12kb) and is remarkably resistant to common inhibitors of DNA polymerases, such as high concentrations of salts, DNA intercalating dyes (SYBR green, SYBR gold, ethidium bromide), organic solvents (phenol), and biological fluids (blood, urine). TOPOTAQ HF has no 5'-3'exonuclease activity. The enzyme mix can be used for high fidelity amplifications of a wide variety DNAs in the 20 kb range.

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

Component	Amount/Cat #	
	H016	H056
TOPOTAQ HF DNA Polymerase	34 µl	170 µl
2X TOPOTAQ HF Amplification Buffer with 6 mM $MgCl_2$*	1.0 ml	5.0 ml
1X TOPOTAQ HF Dilution buffer	100 µl	510 µl

PCR PROTOCOL

The following general procedure is suggested as a guideline and as a starting point when using TOPOTAQ HF 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 ng – 200 ng)**	≥ 1	≥ 1	As required
TOPOTAQ HF (diluted 1:3 in 1X TOPOTAQ 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, 5-40 s	Denature: 94-98°C, 5-40 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 is seen.

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 AND QUALITY CONTROL

One unit of TOPOTAQ HF 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. As determined by direct sequencing of cloned 4.1 kb PCR-amplified GC- reach DNA from *Methanopyrus kandleri* AV19 TOPOTAQ HF DNA polymerase produces three times less errors then mixtures of AmpiTaq and *Pfu* DNA polymerases.

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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