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Taq-*FORCE*™ Long DNA Polymerase

Product No.:	**LTAQ0250**	**LTAQ0500**	
System Includes:		*System Includes:*	
1 x 250 U	Taq-*FORCE*™ Long DNA Polymerase (4 U/µl)	2 x 250 U	Taq-*FORCE*™ Long DNA Polymerase (4 U/µl)
1 x 1 ml	MIGHTY-L™ Buffer (no Mg^{2+}) *(Cat. #MLB0250)*	2 x 1 ml	MIGHTY-L™ Buffer (no Mg^{2+}) *(Cat. #MLB0250)*
1 x 1.2 ml	5X No Smear Additive	2 x 1.2 ml	5X No Smear Additive
1 x 1.2 ml	$MgCl_2$ (50 mM)	2 x 1.2 ml	$MgCl_2$ (50 mM)

Description: Taq-*Force*™ Long DNA polymerase is a high-performance proprietary complex of enzymes and additives specifically designed for applications requiring high processivity and high fidelity. An advanced reaction buffer is supplied for optimal results.

The polymerase is designed for the DNA synthesis of difficult templates such as either long DNA regions or DNA templates with difficult nucleotide composition. Such difficulties may include the premature termination of complementary strand synthesis by misincorporations or pyrophosphorolysis of phosphodiester bonds in the template DNA and the inactivation of the polymerase by the accumulation of pyrophosphate in the reaction mixture and the increased thermal stress (due to longer reaction times in the extension step). The inherent 3'-5' proof-reading activity in the polymerase allows the correction of nucleotide misincorporation, while special additives prevent the undesirable pyrophosphate build up. As compared with other polymerases, the product substantially reduces the non-specific background that appears as a smear.

The polymerase possesses high fidelity and higher thermal stability, which results in better performance than standard Taq polymerase in difficult applications. The product is especially useful for very long DNA regions (>10,000 bp) from trace amounts of template DNA.

The buffer is non-tris based, which provides an optimal pH of 8.8 - 9.2 leading to an improved yield/background ratio.

Unit Definition: One unit is the amount of enzyme that will incorporate 10 nmoles of dNTPs into an acid-insoluble form in 30 minutes at 72°C under the following assay conditions: 25 mM TAPS, pH 9.3 (25°C); 50 mM KCl; 2 mM $MgCl_2$; 0.2 mM each dATP, dGTP, dTTP and 0.1 mM radiolabeled dCTP; 0.25 mg/ml activated salmon sperm DNA; 1 mM â-mercaptoethanol.

Storage Buffer: Enzyme is supplied in 20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 2 mM DTT, 50% glycerol, 0.1% Tween-20.

Storage Conditions: *-20°C. DO NOT STORE IN A FROST-FREE FREEZER.*

Quality Control: Endonuclease, nickase, or exonuclease activities were not detectable after 8 hours incubation, respectively, of 1 µg of lambda, pBR322, or *Hin*d III digested lambda DNA at 72ºC in the presence of 5 units of Taq-*FORCE*™ Long DNA Polymerase.

General Guidelines for Long and Accurate Assays:

Most Critical Parameters

- Use only high MW DNA of the highest available quality
- Avoid freeze-thawing of the template
- The pH of the reaction buffer at 25°C should be 9.0-9.2 (7.9-8.1 at 72°C)
- Check the primer for potential of dimer formation and secondary structure. Ensure the annealing temperature fits the range 65°C-68°C. Redesign the primers if necessary.
- Use longer primers (30-35 mer)
- Reduce the denaturation time to 2-20 sec at 93-94°C

Other Considerations

- Use thin-wall reaction tubes or capillaries
- Elongate at 68°C
- Run less cycles (12-25)
- Perform the reaction in 50 µl volume
- Use filter tips to prepare the reaction mixture
- Overlay the reaction mixture with mineral oil, even in "oil-free" applications
- Always use freshly-prepared primer and dNTP solutions
- Increase the magnesium concentration to 2.0-2.5 mM in 0.2 mM steps.
- For phagemid/cosmid DNA, vary the amount of template DNA in the range of 0.1-5 ng.
- For genomic DNA, vary the amount of template DNA in the range of 0.1 -0.5 µg depending on expected product length.
- Perform a hot start (2 minutes at 94°C)
- Add DMSO (2-5% final conc.) if GC-rich templates are involved. Note that DMSO is highly sensitive to oxidization.

Reaction Conditions:

The optimal conditions (incubation time, temperatures, conc. of enzyme, template DNA, primers, $MgCl_2$) depend on the system and must be determined individually. **IMPORTANT: Spin vials briefly before use.**

Component	**Volume**	**Final Concentration**
10X MIGHTY-L™ Buffer	5 µl	1X
5X No Smear Additive (optional)	variable	0.5-2X
DNTPs Pre-Mixed *(Cat. #DNTP10)*	5 µl	0.25 mM
$MgCl_2$	variable	2.0 – 2.5 mM
Primer	variable	0.1 – 0.2 µM (each)
Taq-*FORCE*™ Long DNA Polymerase	variable	0.04 – 0.08 U/µl
Template DNA	variable	cosmid: 0.1-5 ng, genomic: 0.1-0.5 µg
Sterile H_2O	variable	----------
Final Volume	50 µl	----------

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