MBT060B Taq Polymerase (Recombinant) (3 units/µl)

Components:

Reagents provided	MBT060B			
	500 Units	1000 Units	5000 Units	20 x 500 Units
Taq Polymerase (3 U/µl)	170 µl	335 µl	1.7 ml	3.4ml (20 x 170 µl)
10X HiBuffer A (without $MgCl_2$)	2 ml	4 ml	20 ml	40 ml (20 x 2 ml)
10X HiBuffer S (With 17.5 mM $MgCl_2$)	2 ml	4 ml	20 ml	40 ml (20 x 2 ml)
50mM $MgCl_2$	1 ml	2 ml	10 ml	20 ml (20 x 1 ml)

Description:

Taq DNA Polymerase is a thermostable DNA polymerase of thermophilic bacterium *Thermus aquaticus*. The enzyme catalyzes 5'-3' synthesis of DNA, has no detectable 3'-5' exonuclease (proof-reading) activity, but maintains the 5'-3' exonuclease activity. In addition, Taq DNA Polymerase exhibits deoxynucleotidyl transferase activity, resulting in addition of extra adenines at 3'-end of PCR products.

Features:

- Thermostable enzyme of approximately 94 kDa from *Thermus aquaticus*
- Ultra pure recombinant protein
- Replicates DNA at 74°C and exhibits a half-life of 40 minutes at 95°C
- Generates mostly 3' dA overhang PCR products which are suitable for TA cloning
- DNA sequencing and labeling
- Incorporates modified nucleotides (E.g: biotin, deoxygenin, fluroscently-labelled nucleotides)
- Supplied with two buffers- **10X Hibuffer A** with KCl and **10X Hibuffer S** with $(NH_4)_2SO_4$ [$(NH_4)_2SO_4$ allows for PCR at wide range of Mg^{+2} concentrations and decrease unspecific priming]

Concentration: 3 U/µl

Source: *E.coli* cells with a pol gene from *Thermus aquaticus*

Molecular weight: 94 kDa monomer

Unit Definition:
1U is defined as amount of enzyme that is required to catalyze the incorporation of 10 nmoles of dNTP into acid-insoluble material in 30 minutes at 74°C.

Reaction Buffer:

10X HiBuffer A (Without $MgCl_2$):
500mM KCl, 100mM Tris-HCl (pH 9.1 at 20°C) and 0.1% Triton X -100. The buffer is optimized for use with 0.1 - 0.2mM of each dNTP.

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10X HiBuffer S:
160mM $(NH_4)_2SO_4$, 500mM Tris-HCl (pH 9.2 at 22°C), **17.5mM $MgCl_2$** and 0.1% Triton X-100. The buffer is optimized for use with 0.35mM of each dNTP.

Storage Buffer:
200 mM Tris-HCl (pH 8.0 at 22°C), 100 mM KCl, 0.5% Tween 20, 0.5% Nonidet-P40, 0.1 mM EDTA, 1mM DTT and 50% Glycerol. Store at –20°C

Guidelines for PCR optimization using HiMedia's Taq Polymerase:

- **DNA Template**

  1. Use high quality, purified DNA templates
  2. Approximately 10^4 copies are required to detect the amplification in 25-30 PCR cycles
  3. Use higher DNA concentration when few PCR cycles are desired

- **Primers**

  1.Generally 20-30 bp in size
  2.GC content between 40-60% ideally
  3.Melting temperatures should be between 42-65°C
  4.Final concentration to be used 0.1-0.5µM of each primer

- **Magnesium Concentration**

  1. Ideal for Taq Polymerase is 1.5-2.0mM
  2. Optimum concentration depends on template, buffer and dNTPs
  3. Higher than optimal concentration yields undesired products and if concentration is too low the concentration, no amplification products are detected.

- **dNTPs**

  1. Typical concentration to be used is 200µM
  2. Higher than optimal concentration of dNTPs yields higher yield but low fidelity

- **Taq Polymerase**

  1. Typical concentration to be used is 0.5 to 2 units per 50µl of reaction

- **PCR reaction**

  1. Thaw all reaction components on ice
  2. To PCR reaction, add Taq Polymerase at the end
  3. Once the reaction is set, immediately transfer the tubes to pre-heated thermal Cycler.
  4. Start the reaction with desired cycling conditions with annealing temperature set to 5°C difference of melting temperature between forward and reverse primers.

Quality Control:

All preparations are assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested in DNA amplification.

Buffers recommended for different sizes of template DNA

Buffers	Size of template DNA to be amplified		
	100bp-5kb	5kb-8kb	8kb-20kb
HiBuffer S (1X)	-	+	+
HiBuffer A (1X)	+	-	-
$MgCl_2$	+	+	+

Key: **+** Indicates recommended buffer

Inhibition and Inactivation:

- Inhibitors: ionic detergents (deoxycholate, sarkosyl and SDS) at concentrations higher than 0.06, 0.02, and 0.01% respectively.
- Inactivated by phenol/chloroform extraction.

NOTE:

The error rate for Taq Polymerase, which lacks proof-reading activity is approximately 1 to 2 X 10^{-5}errors (or mutation frequency) per nucleotide per duplication. Accordingly, the accuracy of PCR is 4.5X10^{4}**.** Accuracy is an inverse of the error rate and shows an average number of correct nucleotides incorporated before an error occurs.

Storage conditions: The Taq Polymerase should be stored at -20°C. When stored under the recommended conditions, the product is stable for 2 years.

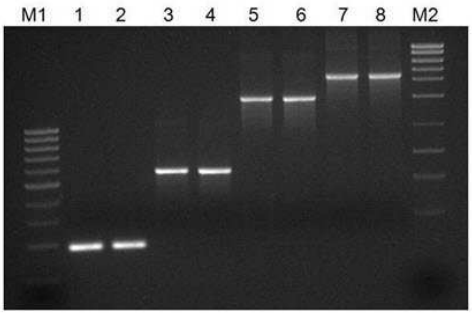

Figure representing amplification of different amplicon sizes using Taq Polymerase with HiBuffer A and HiBuffer S.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.

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