MBT070 Hi-Temp DNA Polymerase

Components

Reagents provided	MBT070			
	100 Units	**200 Units**	**500 Units**	**1000 Units**
Hi-Temp DNA Polymerase (2.5 U/µl)	40 µl	80 µl	200 µl	400 µl
10X HiBuffer A (without $MgCl_2$)	400 µl	800 µl	2 ml	4 ml
10X HiBuffer S (with 17.5 mM $MgCl_2$)	400 µl	800 µl	2 ml	4 ml
50mM $MgCl_2$	200 µl	400 µl	1 ml	2 ml

Description:

Hi-Temp DNA Polymerase is a complex of specific anti-Taq monoclonal antibody with best quality thermostable Taq DNA Polymerase for automatic "hot start" amplification, resulting in greatly enhanced amplification specificity, sensitivity and yield. Hi-Temp DNA Polymerase catalyses the polymerization of nucleotides into duplex DNA in the 5'-3' direction in the presence of Mg^{2+} and has the 5'-3' exonuclease activity.

Features:

- Ultra pure recombinant protein which is reversibly complex with anti-Taq monoclonal antibody that blocks replication activity of the enzyme at moderate temperatures.
- Carefully selected anti-Taq antibodies have high thermal stability, providing protection against non-specific primer extension from room temperature to 70°C.
- Formation of complexes between Taq DNA Polymerase and an anti-Taq antibody forms a basis for automatic "hot start" amplification, which allows for the assembly of amplification reactions at room temperature.
- High stability of the complexes allows for the enormous increase in amplification specificity, sensitivity and yield in comparison to the conventional amplification assembly method.
- Increased specificity as a result of reduced amplification artifacts such as primer-dimer formation and mispriming in multiplex amplification.

Applications:

- High throughput hot start PCR
- RT-PCR
- Highly specific amplification of genomic and cDNA targets up to 3 kb
- Amplification of low copy DNA targets
- Real-time PCR
- Multiplex PCR
- Generation of PCR product for TA cloning

Concentration: 2.5 U/µl

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.

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:
20 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 Hi-Temp DNA 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 Hi-Temp DNA 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.

- **Hi-Temp DNA Polymerase**
  Typical concentration to be used is 0.5 to 2 units per 50µl of reaction.

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.

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

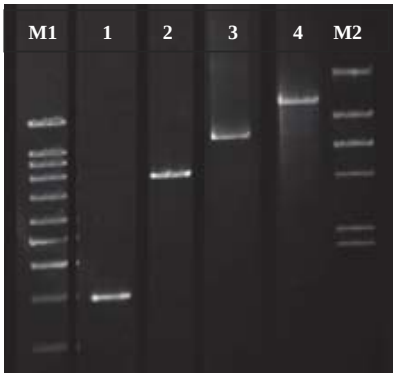

Figure representing amplification of different amplicon sizes using Hi-Temp DNA Polymerase with HiBuffer A and HiBuffer S. Lane M1 : 1kb DNA Ladder, Lane 1 : 1.5kb amplicon, Lane 2 : 5.0kb amplicon, Lane 3 : 8.0kb amplicon, Lane 4 : 10kb amplicon, Lane M2 : Lambda / Hind III Marker

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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