MBT069 Hi- Long Amp DNA Polymerase

Components

Reagents provided	MBT069			
	100 Units	200 Units	500 Units	1000 Units
Hi-Long Amp DNA Polymerase (5 U/µl)	20 µl	40 µl	100 µl	200 µl
10X HiBuffer A (without $MgCl_2$)	400 µl	800 µl	2 ml	4 ml
10X HiBuffer S (with 17.5mM $MgCl_2$)	400 µl	800 µl	2 ml	4 ml
50mM $MgCl_2$	200 µl	400 µl	1 ml	2 ml

Description:

Hi-Long Amp DNA Polymerase is a modified and optimized thermostable enzyme blend containing Taq DNA polymerase, Hi-Long Amp DNA polymerase and enhancing factors for high throughput PCR applications. It ensures higher sensitivity, longer PCR products and higher yields compared to conventional Taq DNA polymerase. It exhibits the 3' to 5' proof reading activity, resulting in considerably higher amplification fidelity than possible with unmodified Taq DNA polymerase.

Features:

- Robust amplification of PCR products
- Excellent for multiplex amplification as it exhibits wider tolerance for Mg^{2+} and salt concentrations, pH, template contaminations and has increased half-life in comparison to unmodified Taq DNA polymerase.
- Improves amplification results with critical templates, such as those containing GC-rich regions, palindromes or multiple repeats.
- Increased amplification product yields and purity.
- Recommended for long targets up to 6 kb from genomic DNA and up to 20 kb from viral DNA.

Applications:

- Routine PCR amplification of DNA fragments up to 6 kb
- RT-PCR
- Genotyping
- Generation of PCR products for TA cloning

Concentration: 5 U/µl

Unit Definition:

1 U is defined as amount of enzyme that required to catalyze the incorporation of 10 nmol 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 Hi-Long Amp 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-Long Amp 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-Long Amp DNA Polymerase**

  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 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.

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

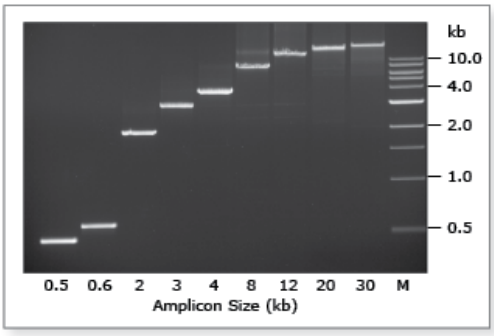

Figure representing amplification of different amplicon sizes using Hi-Long Amp DNA Polymerase with HiBuffer A and HiBuffer S.

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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