MBT088 ChromTaq DNA Polymerase

Components

Reagents provided	MBT088		
	100 Units	200 Units	500 Units
ChromTaq DNA Polymerase (1 U/µl)	100 µl	200 µl	500 µl
10X HiBuffer A (without $MgCl_2$)	400 µl	800 µl	2 ml
10X HiBuffer S (with 17.5 mM $MgCl_2$)	400 µl	800 µl	2 ml
50mM $MgCl_2$	200 µl	400 µl	1 ml

Description:

Taq DNA Polymerase is a thermostable DNA polymerase of thermophillic bacterium *Thermus aquaticus*. It is suitable for applications requiring high temperature synthesis of DNA. 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:

- Color tracer dyes for ease of visualization of the addition of polymerase to the reaction.
- Use of 1 U in every 25 µl final reaction allows direct loading and serves as tracking dyes during gel electrophoresis. The blue and pink color dyes migrate approximately at 4 kb and 0.3 kb respectively on 1% TAE agarose gel.
- 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: 1 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.

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Reaction Buffer:

<u>10X HiBuffer A (Without $MgCl_2$)</u>

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.

<u>10X HiBuffer S</u>

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.

Guidelines for PCR optimization using HiMedia's ChromTaq 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 ChromTaq 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.

- **ChromTaq 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 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 ChromTaq DNA 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 ChromTaq DNA Polymerase should be stored at -20°C. When stored under the recommended conditions, the product is stable for 2 years.

Representative data:

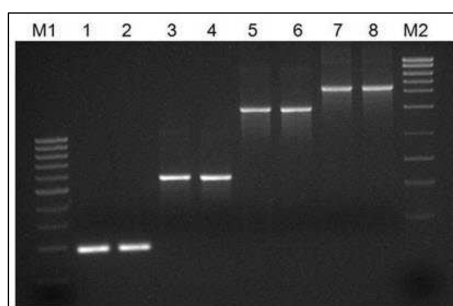

Figure representing amplification of different amplicon sizes using ChromTaq DNA Polymerase with HiBuffer A and HiBuffer S.

Technical Assistance

At HiMedia's, 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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