MBT060D Taq Polymerase (Recombinant) (1 unit/µl)

Components

Reagents provided	Product Code	MBT060D			
		500 Units	1000 Units	5000 Units	20 x 500 Units
Taq Polymerase (1 U/µl)	MBT060D	500 µl	1 ml	5ml	2 ml (20 x 500 µl)
10X HiBuffer A (Without MgCl2)	DS1235	1 ml	2 ml	10 ml	20 ml (20 x 1 ml)
10X HiBuffer S (With 15 mM $MgCl_2$)	DS1234	1 ml	2 ml	10 ml	20 ml (20 x 1 ml)
50mM $MgCl_2$	DS0118	0.5 ml	1 ml	5 ml	10 ml (20 x 0.5 ml)

Description:

Taq DNA Polymerase is a thermostable, recombinant DNA polymerase, which exhibits very high activity in primer extension and other molecular biology applications. The enzyme is isolated from Thermus aquaticus and has a molecular weight of approximately 94 kDa. Taq DNA Polymerase has a 5'→3' DNA polymerase and a 5'→3' exonuclease activity. The enzyme lacks a 3'→5' exonuclease activity (no proofreading ability). Taq DNA Polymerase leaves an A' overhang, which makes the enzyme ideal for TA cloning.

Supplied with two buffers-
10X Hibuffer A with KCl - Promotes high specificity and careful optimization of primer annealing temperatures and Mg2+ concentrations may be required.
10X Hibuffer S with $(NH_4)_2SO_4$ - Ammonium in the buffer minimizes the need for optimization of the MgCl2 concentration or the annealing temperature for most primer-template systems.

Concentration: 1 U/µl

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

Molecular weight: 94 kDa monomer

Unit Definition:
One unit is defined as the amount of polymerase that incorporates 10 nmol of dNTPs into acid-precipitable DNA in 30 minutes at 72 °C under standard assay conditions.

Reaction Buffer:

10X HiBuffer A (Without $MgCl_2$):
100mM Tris-HCl pH 8.5, 500mM KCl, 1% Triton X-100.

10X HiBuffer S (with 15mM $MgCl_2$):
Tris-HCl pH 8.5, $(NH4)_2S0_4$, 15 mM $MgCl_2$, 1% Tween 20®

ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

Registered Office
HiMedia Laboratories Pvt Ltd.
Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Area,
Thane, (West) 400604, Maharashtra, INDIA.
Customer Care No.: 00-91-22-6116 9797
Tel : 00-91-22-6147 1919, 6903 4800
Fax : 6147 1920
Web : www.himedialabs.com
Email : info@himedialabs.com
mb@himedialabs.com

Taq DNA Polymerase in Storage Buffer
5 U/µl Taq, 20 mM Tris-HCl pH 8.5, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween® 20, 50% glycerol.

Protocol

1. Thaw 10X Buffer, dNTP mix and primer solutions. It is important to thaw the solutions completely (some buffers need to reach room temperature) and mix thoroughly before use to avoid localized concentrations of salts. Keep all components on ice. The polymerase is provided in glycerol and does not need thawing. Keep it at -20 °C at all times.

2. Set up reaction mixtures in an area separate from that used for DNA preparation or product analysis. Work on ice at all times.

3. Prepare a master mix according below table. The master mix typically contains all the components needed for extension except the template DNA.

Reaction components (master mix and template DNA)

Component	Vol./reaction*	Final concentration*
10X Buffer	5 µl	1x
25 mM $MgCl_2$	0 µl (0 – 6 µl)	1.5 mM (1.5 – 4.5 mM)
dNTP mix (10mM mM each)	1 µl	0.2 mM of each dNTP
Forward Primer (10 µM)	1 µl (0.5 – 5 µl)	0.2 µM (0.1 – 1.0 µM)
Reverse Primer (10 µM)	1 µl (0.5 – 5 µl)	0.2 µM (0.1 – 1.0 µM)
Taq DNA Pol.	1 µl (1 –5 µl)	1 unit (1 – 5 units)
Molecular Biology Grade Water for PCR	X µl	-
Template DNA	X µl	genomic DNA: 50 ng (10 – 500 ng) plasmid DNA: 0.5 ng (0.1 – 1 ng) bacterial DNA: 5 ng (1 – 10 ng)
TOTAL volume	50 µl	-

* Suggested starting conditions; theoretically used conditions in brackets. The final volume can be reduced to 25 µl by using half of the volumes suggested in Vol./reaction, eg. 0.1 µl Taq instead of 0.2 µl Taq.

4. Mix the master mix thoroughly and dispense appropriate volumes into reaction tubes. Mix gently, e.g. by pipetting the master mix up and down a few times.

5. Add template DNA to the individual tubes containing the master mix.

6. Program the thermal cycler according to the manufacturer's instructions.

For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.

7. Place the tubes in the thermal cycler and start the reaction.

Cycles	Duration of cycle	Temperature
1	2 – 5 minutes[a]	95 °C
25 – 35	20 – 30 seconds[b] 20 – 40 seconds[c] 30 seconds[d]	95 °C 50 – 65 °C 72 °C
1	5 minutes[e]	72 °C

[a.] Initial denaturation step (optional).

[b.] Denaturation step: This step is the first regular cycling event and consists of heating the reaction to 95 °C for 20 – 30 seconds. It causes melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.

[c.] Annealing step: The reaction temperature is lowered to 50 – 65 °C for 20 – 40 seconds allowing annealing of the primers to the single- stranded DNA template. Typically, the annealing temperature is about 3 – 5 °C below the Tm (melting temperature) of the primers used.

[d.] Extension/elongation step: Taq polymerase has its optimum activity temperature at 72 °C. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand. The extension time depends on the length of the DNA fragment to be amplified. As a rule of thumb, at its optimum temperature the DNA polymerase will polymerize a thousand bases per minute.

[e.] Final elongation: This single step is occasionally performed at a temperature of 72 °C for 5 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended.

Note: If 15 mM $MgCl_2$ is present in 10x PCR Buffers, the 1x concentration is 1.5 mM $MgCl_2$. In some applications, more than 1.5 mM $MgCl_2$ is required for best results (See below table).

Additional volume (µl) of $MgCl_2$ per 50 µl reaction

Final $MgCl_2$ conc. in reaction (mM)	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Volume of 25 mM $MgCl_2$ (µl)	0	1	2	3	4	5	6

Quality Control:

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

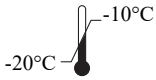
NOTE:

The error rate for Taq Polymerase, which lacks proof-reading activity is approximately 1 to 2 $X10^{-5}$ errors (or mutation frequency) per nucleotide per duplication. Accordingly, the accuracy of PCR is 4.5 X 10^4**.** 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.

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.



Storage temperature

Do not use if package is damaged

HiMedia Laboratories Private Limited,
Reg. Off: Plot No. C-40, Road No. 21Y,
MIDC, Wagle Industrial Area, Thane,
(West) 400604, Maharashtra, INDIA.
www.himedialabs.com

05/2025

PIMBT060D_O/0522 MBT060D-03