HI-FI POP DNA POLYMERASE

With 5x HI-FI Buffer (7.5 mM $MgCl_2$)

Concentration: 2 units/µl

Cat. No.: 257653
500 Units

MADE IN **DENMARK**

-	Hi-Fi Pop DNA Polymerase	5x HI-FI Buffer, 7.5 mM $MgCl_2$	$MgCl_2$ 25 mM
ID No.	**CL0.250-0045**	**CL1.500-0049**	**CL1.500-0047**
Cap colour	**Black**	**Clear**	**Clear**
Content	250 µl	4 x 1.5 ml	1.5 ml

Key Features

- High fidelity: > 60x Taq[1)]
- Long range amplification: 18 kb human genomic DNA
- High elongation rate: 10 sec/kb
- Excellent performance on a vast range of amplicons (high AT and high GC)
- Recommended for cloning, mutagenesis and other molecular applications requiring extremely high fidelity

Hi-Fi Pop DNA Polymerase is a thermostable, chimeric DNA Polymerase created specifically for low-bias, high fidelity amplification of a vast range of amplicons. Hi-Fi Pop DNA Polymerase delivers high-speed elongation and processivity, due to its fusion with a DNA-binding domain.

[1)] Determined through a novel NGS-based analysis of nucleotide misincorporation during PCR

Protocol

Optimal reaction conditions such as incubation times, temperatures and amount of template DNA may vary and must be determined individually. Amplification of templates with high GC content, extensive secondary structures as well as long range amplification may require more optimization - for tips see section *Strategies for Optimization*.

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

1. Thaw 5x HI-FI Buffer, dNTP mix and primer solutions. **A precipitate is often seen in the 5x HI-FI Buffer after thawing. It is recommended to completely thaw and thoroughly mix the buffer to ensure proper resuspension of precipitates.**
2. Prepare a master mix according to Table 1. The master mix typically contains all the components needed for amplification except the template DNA. It is important to add Hi-Fi Pop DNA Polymerase last to prevent primer degradation caused by the 3'→5' exonuclease activity.
3. 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.
4. Add template DNA to the individual tubes containing the master mix.
5. Program the thermal cycler according to Table 2. For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.
6. Place the tubes in the thermal cycler and start the reaction.

Table 1. Recommended reaction components

Component	Vol./reaction*	Final concentration*
5x HI-FI Buffer	5 µl	1x
dNTP mix (10 mM each)	0.5 µl	0.2 mM of each dNTP
Primer A (10 µM)	0.5 µl	0.2 µM
Primer B (10 µM)	0.5 µl	0.2 µM
25 mM $MgCl_2$	0 µl (0 – 3 µl)	1.5 mM (1.5 – 4.5 mM)
HI-FI HiFi DNA Pol. 2U/µl	0.25 µl (0.125 – 0.5 µl)	0.5 units (0.25 – 1 units)
Betaine (5M)**	5 - 10 µl	1 - 2M
PCR-grade H_2O	X µl	-
Template DNA	X µl	genomic DNA: 20 ng (10 – 500 ng) plasmid DNA: 0.5 ng (0.1 – 1 ng) bacterial DNA: 5 ng (1 – 10 ng)
TOTAL volume	25 µl	-

* Suggested starting conditions; theoretically used conditions in brackets.
** Suggested for GC-rich amplification and long-range amplification. See section *Strategies for Optimization.*

Table 2. Three-step PCR program

Step	Duration of cycle	Temperature
Initial denaturation	2 min[a)]	98 °C
25 – 35 cycles	10 – 20 sec[a)] 15 – 30 sec[b)] 10 – 60 sec[c)]	98 °C 55 – 70 °C 72 °C
Final elongation	5 min	72 °C

[a.] Denaturation: 2 min initial denaturation is sufficient for most templates. During thermocycling, 10 seconds usually works very well. Longer denaturation times might be required for long range PCR or amplification from templates with a high GC content.

[b.] Primer annealing: Typically, the annealing temperature is about 3 – 5 °C below the T_m (melting temperature) of the primers used. **Because of the high salt content within the 5x HI-FI Buffer, annealing temperature will likely be higher than with more traditional PCR buffers.**

[c.] Extension: The recommended extension temperature is 72°C. Extension times highly depends on the length of the amplicon. **Generally, we recommend an extension time of 10-30 seconds per kb for complex genomic targets. 10 seconds per kb is often sufficient for simpler targets (such as plasmid) or short complex targets (< 3 kb). 30-60 seconds per kb is recommended for long amplicons (> 3 kb).**

Strategies for Optimization

Long-range amplification

- Longer extension times often resolve low-yield amplification of long amplicons.
- Increased amount of Hi-Fi Pop DNA Polymerase (up to 1U) have often resolved low-yield reactions from very long targets (>8 kb)
- Increased dNTP concentration (up to 1.6 µM) often increases yield and decreases unspecific product creation.
- The addition of 1-2 M Betaine solution often improves reaction performance (See Additional Products for ordering information).
- Increased template concentration will increase product yield.
- Increased primer concentration can increase product yield for some reactions.

GC-rich amplification

- The addition of 1 - 2 M Betaine solution often improves reaction performance (See Additional Products for ordering information).

Primers

- Primers of 20 – 40 nucleotides with a GC content of 40 - 60 % are recommended. Online Software such as the Primer3plus

https://primer3plus.com/cgi-bin/dev/primer3plus.cgi can be used to design primers.

$MgCl_2$

- The optimal $MgCl_2$ concentration should be determined empirically but in most cases a concentration of 1.5 mM, as provided in the common 1x HI-FI Buffer, will produce satisfactory results. Table 3 provides the volume of 25 mM $MgCl_2$ to be added to the master mix if a higher $MgCl_2$ concentration is required.

Table 3. Additional volume (µl) of $MgCl_2$ per 25 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 $MgCl_2$	0	0.5	1	1.5	2	2.5	3

Kit Components

- **2U/µl Hi-Fi Pop DNA Polymerase in Storage Buffer**
  50 mM Tris-HCl pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1.0 mM DTT, 0.1% Tween® 20, 50% Glycerol

- **5x HI-FI Buffer (7.5 mM $MgCl_2$)**

- **25 mM $MgCl_2$**
  For eventual optimization of PCR conditions.

5 M Betaine Enhancer Solution
Sold separately. Cat No.: A351104

More info

Recommended Storage and Stability
Long term storage at -20 °C. Product expiry at -20 °C is stated on the label.
Optional: Store at +4 °C for up to 6 months.

Quality Control
Hi-Fi Pop DNA Polymerase is tested for contaminating activities with no traces of endonuclease activity or nicking activity. Furthermore, long range capacity is tested on human gDNA target of 18 kb.

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

For Research Use Only. Not for use in diagnostics procedures.

Other product sizes, combinations and customized solutions are available. Please look at www.dutscher.com or ask for our complete product list for PCR Enzymes. For customized solutions please contact us.

Made in Denmark

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