HI-FI POP DNA POLYMERASE
2x Master Mix

MADE IN DENMARK

Cat. No.: 257656
500 Reactions

-	Hi-Fi Pop DNA Polymerase 2x Master Mix 3 mM $MgCl_2$
ID No.	**CL1.250-0046**
Cap colour	**Blue**
Content	10 x 1.25 ml

Key Features

- Convenient reaction set-up
- High fidelity: > 60x Taq[1)]
- Long range amplification: 11 kb for gDNA
- 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 2x Master Mix is an all-in-one 2x master mix containing the Hi-Fi Pop DNA Polymerase, HI-FI Buffer, dNTPs and $MgCl_2$. Simply mix Hi-Fi Pop DNA Polymerase 2x Master Mix with primers, DNA template and water and you are ready to carry out PCR.

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, high secondary structures as well as long range amplification may require more optimization – for tips see section *Strategies for Optimization*

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

1. Thaw Hi-Fi Pop DNA Polymerase 2x Master Mix and primer solutions.
   It is recommended to completely thaw and thoroughly mix the master mix to ensure proper resuspension of precipitates.
2. Prepare the reaction mix. Table 1 shows the reaction set up for a final volume of 50 µl. If desired, the reaction size may be scaled down.
3. Mix the reaction 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 reaction mix.
5. 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.
6. Place the tubes in the thermal cycler and start the reaction.

Table 1. Reaction components

Component	Vol./reaction*	Final concentration*
2x Master Mix	25 µl	1x
Primer A (10 µM)	1 µl	0.2 µM
Primer B (10 µM)	1 µl	0.2 µM
25 mM $MgCl_2$	0 µl (0 – 6 µl)	1.5 mM (1.5 – 4.5 mM)
Betaine (5M)**	10 - 20 µl	1 – 2M
PCR-grade H_2O	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.
** Suggested for GC-rich amplification and long-range amplification. See section *Strategies for Optimization*.

Table 2. Three-step PCR program

Cycles	Duration of cycle	Temperature
1	2 min[a)]	98 °C
25 - 35	10 – 20 sec[a)] 15 – 30 sec[b)] 10 – 60 sec[c)]	98 °C 55 – 70 °C 72 °C
1	5 minutes	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 Hi-Fi Pop DNA Polymerase 2x Master Mix, annealing temperature will likely be higher than with more traditional PCR master mixes.**

[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.
- 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

- 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 final concentration of 1.5 mM, as provided in Hi-Fi Pop DNA Polymerase 2x Master Mix, 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 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 $MgCl_2$	0	1	2	3	4	5	6

Kit Components

Hi-Fi Pop DNA Polymerase 2x Master Mix

- Hi-Fi Pop DNA Polymerase
- Optimized buffer components, 3.0 mM $MgCl_2$
- dNTPs

5 M Betaine Enhancer Solution

Sold separately. Cat No.: 257543

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 6 kb.

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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