Fidelio® Hot Start Master Mix

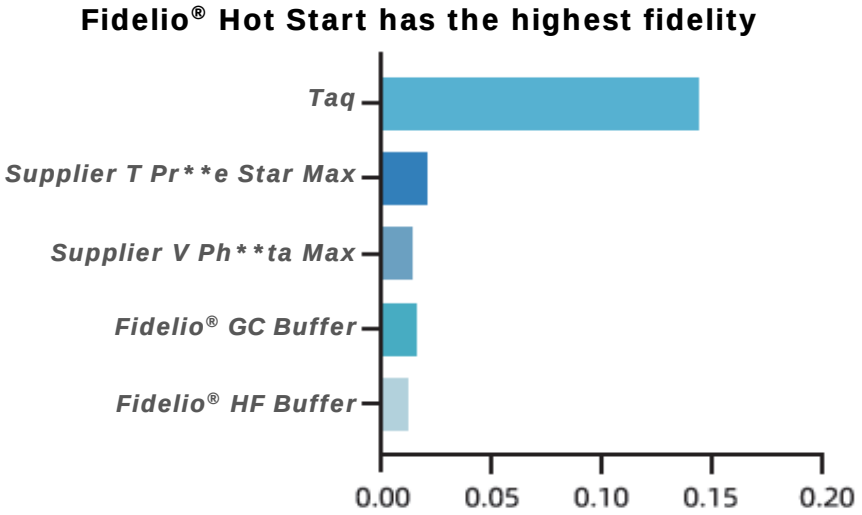
20 rxn / 50 rxn / 200 rxn (50 µL/rxn)

Reference: OZYA009-20 / OZYA009-50 / OZYA009-5 (anciennement OZYA009-200)

VOLUME: 500 µL / 1.25 mL / 4 x 1.25 mL
CONCENTRATION: 2x
STORAGE: -20°C upon arrival
SHELF-LIFE: one year from the date of reception when stored properly

Product Description:

The Fidelio® Hot Start DNA polymerase is a novel engineered high-fidelity enzyme with exceptional processivity. The Fidelio® has a unique enhancement domain that improves the fidelity and the extension speed. It has also a built-in antibody-mediated hot-start system that inhibits efficiently non-specific amplifications at room temperature. The Fidelio® Hot Start DNA polymerase has strong 3'-5' exonuclease activity (proofreading activity), thus it offers extreme high fidelity (see the fidelity comparison figure below). Fidelio® Hot Start DNA Polymerase produces blunt ends.



Fidelity comparison: mutation rate was measured by sequencing for each of the tested enzymes. The results show that Fidelio® Hot Start DNA Polymerase gives the lowest mutation frequency.

Fidelio® Hot Start Master Mix is a 2x master mix that includes all components, such as Fidelio® Hot Start DNA polymerase, dNTPs, reaction buffer and optimized $MgCl_2$ for PCR experiments. Template DNA and target-specific primers are user provided. Fidelio® Hot Start Master Mix gives good amplification efficiency for a variety of templates like genomic DNA from human, animals, plants … or cDNAs.

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List of Components:

Component	OZYA009-20	OZYA009-50	OZYA009-200
Fidelio® Hot Start Master Mix (2x)	500 µL	1.25 mL	4 x 1.25 mL

Precautions:

1. Prepare all reaction components on ice, then quickly transfer the reaction mixture to a thermocycler preheated at 98 °C.
2. All components should be mixed well and collected at the bottom of a PCR tube by a quick spin before use.
3. Add Fidelio® Hot Start Master Mix to the reaction mixture at last to prevent primer degradation by its strong 3'-5' exonuclease activity.
4. **Note:** The Fidelio® Hot Start DNA polymerase requires **special reaction conditions** that are different from other polymerase protocols. Please **refer to the "standard protocol" section** below for optimal amplification yields.

Standard protocol:

1. **Prepare the following reaction mix on ice**:

Reagent	20 µL	50 µL	Final Concentration
Forward Primer (10 µM)	0.4 µL	1 µL	0.2 µM
Reverse Primer (10 µM)	0.4 µL	1 µL	0.2 µM
DNA template	variable	variable	< 300 ng/rxn*
Nuclease free water	to 20 µL	to 50 µL	-
Fidelio® Hot Start Master Mix 2x	10 µL	25 µL	1x

Note*: the optimal concentration varies with DNA templates. Please refer to the "PCR considerations and optimizations**" section below.*

2. **Set the PCR cycling program*** :

Stage 1	Pre-denaturation	1 cycle	98 °C	45 sec - 3 min
Stage 2	Cycles	25-35 cycles	98 °C	10 sec
			55 – 65 °C	20 - 30 sec
			72 °C	10 - 30 sec/kb
Stage 3	Final extension	1 cycle	72 °C	1 - 5 min
	Hold	1 cycle	4 – 12 °C	∞

***Note*: the optimal PCR cycling program varies with DNA templates and amplicon targets. Please refer to the "**PCR considerations and optimizations**" section below.*

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PCR considerations and optimizations:

Template:

Ultra-pure DNA templates are important for high-fidelity PCR reactions. The recommended DNA amounts of different templates are described in the table below:

DNA template type	Input amount for 50 µL reaction mixture
Genomic DNA (human, animals, or plants)	10 ng – 300 ng
Genomic DNA (*E. coli*, lambda genome)	10 ng – 100 ng
Plasmid DNA	1 pg – 10 ng
cDNA (reverse transcription reaction mixture)	< 5 µL (less than 10 % of the final reaction volume)

Note: if long fragments are amplified, the input amount of template should be increased appropriately.

Primers:

Design oligonucleotide primers of 20 - 40 nucleotides in length with a GC content of 40 - 60%. Primers can be designed and analyzed using software such as Primer 3. The final concentration of each primer in the PCR reaction should be in the range of 0.2 - 1 µM.

PCR cycling:

Denaturing – a pre-denaturation of 45 seconds at 98 °C allows complete denaturing of most of the DNA templates. When using high-complexity DNA templates, the pre-denaturation time should be extended up to 3 minutes for fully denaturation. For low-complexity DNA templates, the general denaturation condition is 5 - 10 seconds at 98 °C.

Annealing – **IMPORTANT** the **optimal annealing temperature** for Fidelio® Hot Start DNA polymerase **is usually higher** than other PCR polymerases. The general rules are:

a) primers **> 20 nt**: the annealing temperature is the **lower primer Tm+3°C**.
b) primers **< 20 nt**: the annealing temperature is the **lower primer Tm**.

For new primer sets, we recommend a gradient PCR to determine the optimal annealing temperature. For two-step amplification protocol (2-step PCR), the annealing temperature should be set to the extension temperature.

Extension - the recommended extension temperature of Fidelio® is 72°C. The extension time depends on the length and the complexity of the templates. For low-complexity templates such as plasmid DNA, the extension time is 10-30 sec/kb. For high-complexity templates, such as human genomic DNA, it is necessary to increase the extension time to 1 min/kb. For cDNA templates, the extension time should be set to < 1 min/kb in some cases.

Cycles - to obtain enough yield of PCR products, 25-35 cycles are recommended.

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Cloning of PCR Products:

Blunt-end cloning: Fidelio® Hot Start DNA polymerase generates blunt-end PCR products that can be directly used in the downstream blunt-end cloning.

T/A cloning: the PCR products should be purified to remove Fidelio® Hot Start DNA polymerase before d(A) tailing reaction. Since the 3'-5' exonuclease activity of Fidelio® Hot Start DNA polymerase will remove the d(A)-overhangs. *Taq* DNA polymerase (OZYME, OZYA001-1000) or *Klenow* Exo$^-$ are recommended to d(A) tailing reaction of the purified PCR products.

FOR ORDERING:

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Document version :

OZYA009-1020

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