

## Fidelio® Hot Start PCR Kit

20 rxn / 50 rxn / 200 rxn (50 µ/rxn)

Reference: OZYA010-20 / OZYA010-50 / OZYA010-200

**SIZE:** 40 u / 100 u / 400 u

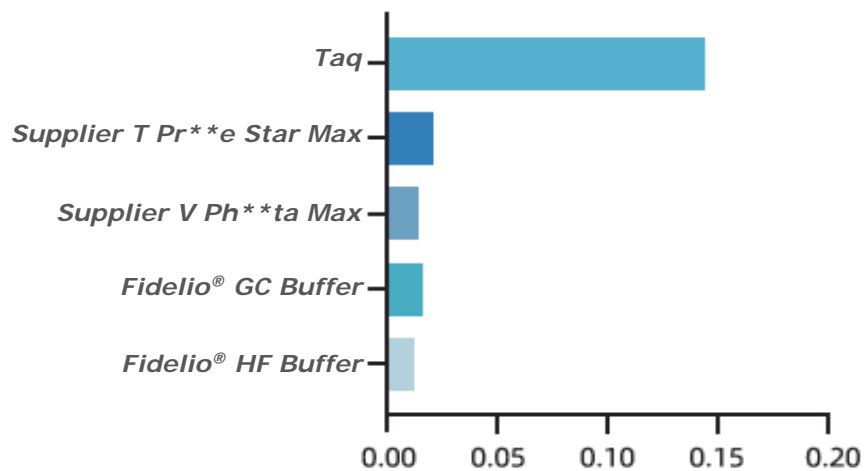
**STORAGE:** -20°C upon arrival

**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.

### Fidelio® Hot Start has the highest fidelity



**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.

The Fidelio® Hot Start PCR Kit is supplied with 5x Fidelio® HF Buffer, 2.5x Fidelio® GC Buffer and ultra-pure dNTP mix. The 5x Fidelio® HF Buffer is optimized for standard high-fidelity amplifications while the 2.5x Fidelio® GC Buffer is recommended for the amplifications of complex and/or GC-rich templates.

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## List of Components :

Component	OZYA010-20	OZYA010-50	OZYA010-200
Fidelio® Hot Start DNA Polymerase (2 u/µL)	20 µL	50 µL	200 µL
5x Fidelio® HF Buffer	200 µL	500 µL	2 X 1 mL
2.5x Fidelio® GC Buffer	400 µL	1 mL	4 X 1 mL
dNTP Mix (10 mM each)	20 µL	50 µL	200 µL

## 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 DNA polymerase 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:

a) 5x Fidelio® HF Buffer reaction mixture

Reagent	20 µL	50 µL	Final Concentration
5x Fidelio® HF Buffer	4 µL	10 µL	1x
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*
dNTP (10 mM)	0.4 µL	1 µL	0.2 mM
Fidelio® DNA Polymerase (2 u/µl)	0.4 µL	1 µL	2 u / 50 µl
DNase/RNase free water	to 20 µL	to 50 µL	-

b) 2.5x Fidelio® GC Buffer reaction mixture

Reagent	20 µL	50 µL	Final Concentration
2.5x Fidelio® GC Buffer	8 µL	20 µL	1x
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*
dNTP (10 mM)	0.4 µL	1 µL	0.2 mM
Fidelio® DNA Polymerase (2 u/µl)	0.4 µL	1 µL	2 u / 50 µl
DNase/RNase free water	to 20 µL	to 50 µL	-

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

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## 2. Set the PCR cycling program:

<b>Stage 1</b>	Pre-denaturation	1 cycle	98 °C	45 sec - 3 min
<b>Stage 2</b>	Cycles	25-35 cycles	98 °C	10 sec
			55 – 65 °C	20 - 30 sec
			72 °C	10 - 30 sec/kb
<b>Stage 3</b>	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.

## PCR considerations and optimizations:

### Template:

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

DNA template type	Input amount for <b>50 µL</b> reaction mixture
Genomic DNA (human, animals, or plants)	10 ng – 300 ng
Genomic DNA ( <i>E. coli</i> , 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.

### Reaction buffer & enhancers:

The Fidelio® Hot Start PCR Kit contains a Fidelio® 5x HF Buffer and a 2.5x Fidelio® GC Buffer. The 5x Fidelio® HF Buffer is designed for standard high-fidelity PCR amplification and the 2.5x Fidelio® GC Buffer is optimized for GC-rich templates. Enhancers may help increasing the amplification efficiency for problematic templates, like high GC content or strong secondary structure genes. Note: enhancers are already included in the 2.5x Fidelio® GC Buffer. It is not recommended to add any additional enhancers to this Buffer. Excess amount of enhancer may be inhibitory.

### 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.

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**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 yields of PCR products, 25-35 cycles are recommended.

#### **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 (OZYA001-1000, OZYME) or *Klenow Exo<sup>-</sup>* are recommended to d(A) tailing reaction of the purified PCR products.

#### **FOR ORDERING:**

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**PRODUCT FOR RESEARCH USE ONLY**

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