Owi AptaFi Hot Start Master Mix

User Manual

References: 258513 - 258514 - 258515

FOR RESEARCH USE ONLY

SIZE: 20 / 100 / 500 Rxns of 50 µl PCR – 1 x 0.5 mL / 2 x 1.25 mL / 10 x 1.25 mL

STORAGE TEMPERATURE: Store at -20°C. The product can be stored at 4 °C for 1 month.

SHELF LIFE: one year from the date of reception when stored properly.

PRODUCT DESCRIPTION:

Owi AptaFi Hot Start Master Mix is a high-fidelity enzyme optimized for demanding PCR applications requiring both accuracy and robustness. Derived from a proofreading Pfu DNA polymerase, **Owi AptaFi Hot Start** combines 3'–5' exonuclease activity with engineered mutations that enhance processivity and DNA binding, allowing efficient amplification of long or complex targets.

The enzyme generates blunt-end products and is ideal for high-fidelity PCR, difficult templates, high-specificity reactions, and applications such as site-directed mutagenesis, gene editing, and NGS library preparation. Its performance makes it particularly suitable for multiplex and high-throughput assays.

Hot start functionality is provided by an aptamer-based reversible inhibition system, ensuring maximal specificity and preventing non-specific amplification at room temperature. **Owi AptaFi Hot Start** enables fast extension rates (10-30 s/kb) and high yields, even on templates exceeding 17 kb.

LIST OF COMPONENTS:

Component	OZYA014-20	OZYA014-100	OZYA014-500
2X Owi AptaFi Hot Start Master Mix	0,5 mL	2 x 1.25 mL	10 x 1.25 mL

2X Owi AptaFi Hot Start Master Mix contains 6 mM mgCl2, 2 mM dNTPs, stabilizers and enhancers. Further addition of PCR enhancers or MgCl2to the reaction is not recommended

DOMINIQUE DUTSCHER SAS

Dominique DUTSCHER SAS - 67170 BERNOLSHEIM - FRANCE
Tel.: +33 3 88 59 33 90 – www.dutscher.com
Customer Service – Order: info@dutscher.com
Technical Support: demandes_techniques@dutscher.com

Standard Protocol:

1. **Prepare the following reaction mix**

Reagent	25 µL reaction	50 µL reaction	Final concentration
2x Owi AptaFi Hot Start Master Mix	12.5 µL	25 µL	1x
Forward primer (10 µM)	1 µL	2 µL	0,4 µM
Reverse primer (10 µM)	1 µL	2 µL	0,4 µM
Template DNA	Variable	variable	variable
PCR grade dH2O	Up to 25 µL final volume	Up to 50 µL final volume	-

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

Stage 1	Pre-denaturation	1 cycle	95 °C	1 min
Stage 2	Denaturation	25 – 35 cycles	98 °C	15 sec
	Annealing		60 – 75 °C	15 sec
	Extension		72 °C	10 - 30 sec/kb

PCR CONSIDERATIONS AND OPTIMIZATIONS:

Template:
The recommended DNA template amounts of different templates are described in the table below:

DNA template type	Input amount for 25 µL reaction mixture*	Input amount for 50 µL reaction mixture*
Genomic DNA (human, animals, or plants)	2,5 ng – 250 ng	5 ng – 500 ng
Plasmid or viral DNA	2.5 pg – 250 pg	5 pg – 500 pg

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

Primers:

Primers of 20 – 40 nucleotides with a GC content of 40 - 60 % are recommended. 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 – 0.6 µM.

DOMINIQUE DUTSCHER SAS

Dominique DUTSCHER SAS - 67170 BERNOLSHEIM - FRANCE
Tel.: +33 3 88 59 33 90 – www.dutscher.com
Customer Service – Order: info@dutscher.com
Technical Support: demandes_techniques@dutscher.com

PCR cycling:

Denaturating

Denaturation should be performed during 1 min at 95 °C. for more complex templates, increasing the temperature to 98-100°C is recommended.

Annealing

IMPORTANT: the optimal annealing temperature for **Owi AptaFi Hot Start Master Mix** is usually higher than other PCR polymerases, so we recommend a gradient PCR to determine the optimal temperature. The general rule, 4x (G+C) + 2x (A+T) °C, could be used as a starting point.
For the two-step amplification protocol (2-step PCR), the annealing temperature should be set between 65 and 72°C and eventually adjusted based on the results.

Extension

The recommended extension temperature of **Owi AptaFi** is 72°C. The extension time depends on the length and the complexity of the templates.
For most applications 30 sec/kb is recommended. Shorter extension time (down to 10 sec/kb) is possible.
Two-step PCR is possible by combining annealing and extension at 68 to 75°C.

Multiplex PCR

The optimal extension time in multiplex PCR depends on template complexity, amplicon length, and the number of targets. We recommend basing the initial extension time on the longest expected fragment, then increasing in 10 to 30 second increments if additional optimization is required.

Cycles number

25-35 cycles are recommended.

Gel visualization

As the Hot Start technology of Owi AptaFi is based on an aptamer, a band of approximately 40 bp may appear on agarose gels. This band should not be mistaken for non-specific amplification.

DOMINIQUE DUTSCHER SAS

OZYME SAS - 78210 SAINT-CYR-L'ECOLE - FRANCE
Tel.: +33 1 34 60 24 24 - www.ozyme.fr/info
Customer Service - Order: commande@ozyme.fr
Technical Support: tech@ozyme.fr

Dominique DUTSCHER SAS - 67170 BERNOLSHEIM - FRANCE
Tel.: +33 3 88 59 33 90 – www.dutscher.com
Customer Service – Order: info@dutscher.com
Technical Support: demandes_techniques@dutscher.com