Owi Taq Master Mix Ready to Load

User manuel

References: 258504 - 258505 - 258506

FOR RESEARCH USE ONLY

SIZE: 40 / 200 / 1000 Rxns of 50 µL PCR - 1 mL / 1 x 5 mL / 5 x 5 mL

STORAGE TEMPERATURE: Store at-20°C.

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

PRODUCT DESCRIPTION:

Owi Taq Master Mix Ready to Load is an optimized premix including all components necessary for conventional PCR amplification except the DNA template and primers. The **Owi Taq Master Mix Ready to Load** is delivered in 2X and includes DNA polymerase, dNTPs, MgCl2, KCl, stabilizers and loading dye. The template DNA may be purified DNA as well as bacterial colonies or liquid culture, crude extract, or cDNA, etc.
This 2X PCR mix can amplify target up to 10 kb from complex genomic DNA such as human genomic DNA or up to 20 kb from simple template such as lambda DNA. It is ideal for multiple applications such as standard PCR, colony PCR for vector construction identification, direct PCR for genotyping, etc.

ENZYME SOURCE:

The DNA polymerase gene in the Owi Taq Master Mix Ready to Load was produced in E. coli and purified by multiple separation steps and has the following enzymatic properties:

- Heat inactivation: no
- 5'-3' exonuclease activity: no
- 3'-5' exonuclease activity: yes
- Fidelity: 6x Taq
- Product ends: blunt ends

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LIST OF COMPONENTS:

Component	OZYA011-40	OZYA011-200XL	OZYA011-1000
Owi Taq Master Mix Ready to Load (2X)	1 mL	1 x 5 mL	5 x 5 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 the tube by a quick spin before use.
3. Add **Owi Taq Master Mix Ready to Load** to the reaction mixture at last to prevent primer degradation by its 3´-5' exonuclease activity.

STANDARD PROTOCOL:

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

Reagent	20 µL	50 µL	Final Concentration
Owi Taq Ready to Load 2X	10 µL	25 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*
DNase/RNase free water	to 20 µL	to 50 µL	-

***Note**: The optimal reaction concentration varies with different 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
Stage 2	Cycles	30 cycles	98 °C	10 sec
			55 – 65 °C	30 sec
			72 °C	20 - 30 sec/kb
Stage 3	Final extension	1 cycle	72 °C	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:
The recommended DNA template 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 – 100 ng
Genomic DNA (*E. coli*, lambda genome)	500 pg – 200 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 0.1 - 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 Owi Taq Master Mix Ready to Load **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

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The recommended extension temperature of **Owi Taq Master Mix Ready to Load** 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 sec/kb. For high-complexity templates, such as human genomic DNA, it is necessary to increase the extension time to 20 – 30 sec/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.

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