Owi Taq Hot Start Master Mix Ready to Load

User Manual

References: OZYA013-20 / OZYA013-100 / OZYA013-500 / OZYA013-1000

FOR RESEARCH USE ONLY

SIZE: 20 / 100 / 500 / 1000 Rxns of 50 µl PCR

1 x 0.5 mL / 2 x 1.25 mL / 10 x 1.25 mL / 20 x 1,25 mL

STORAGE TEMPERATURE: Long term storage at -20 °C. Store at +4 °C for up to 6 months.

SHELF LIFE: Product expiry at -20 °C is stated on the label.

PRODUCT DESCRIPTION:

Owi Taq Hot Start Master Mix Ready to Load is a 2x all-in-one master mix containing Owi Taq Hot Start DNA Polymerase, an ammonium-based buffer system, dNTPs, $MgCl_2$, a stabilizer, and an inert blue tracking dye.
There is no need to add separate loading dyes: PCR products can be directly loaded onto an agarose gel for electrophoresis and visualization.
The blue dye migrates at approximately 400–500 bp on a 0.5–1.5% agarose gel, depending on gel conditions.
Owi Taq Hot Start DNA Polymerase is a chemically modified version of Taq polymerase. A temperature-sensitive blocking group is bound to the active site, keeping the enzyme inactive at room temperature. Heat activation during the initial denaturation step removes this blocking group, allowing polymerase activity to begin.

This hot-start mechanism prevents non-specific amplification and primer-dimer formation during reaction setup, resulting in higher specificity and improved yields compared to standard Taq polymerase.

COMPONENTS:

- Tris-HCl pH 8.5, $(NH_4)_2S0_4$, 3.0 mM $MgCl_2$, 0.2% Tween® 20
- 0.4 mM of each dNTP
- Owi Taq Hot Start Polymerase
- Inert blue dye and stabilizer

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Components	OZYA013-20	OZYA013-100	OZYA013-500	OZYA013-1000
Owi Taq Hot Start Master Mix Ready to Load 2X	0,5 mL	2 x 1.25 mL	10 x 1.25 mL	20 x 1.25 mL

QUALITY CONTROL:

Owi Taq Hot Start Polymerase is tested for contaminating activities, with no traces of endonuclease activity, nicking activity or exonuclease activity.

PROTOCOL:

This protocol serves as a guideline to ensure optimal PCR results when **using Owi Taq Hot Start Master Mix Ready to Load**. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be determined individually.

1. Thaw the Master Mix and primer solutions. It is important to thaw the solutions completely and mix thoroughly before use to avoid localized concentrations of salts. **Important:** Spin vials briefly before use.

2. Prepare the reaction mix. Table 1 shows the reaction mix set up for a final volume of 50 µl.

Table 1. Reaction mix and template DNA

* Suggested starting conditions; theoretically used conditions in brackets.

Reagent	20 µL	50 µL	Final Concentration
Owi Taq Hot Start 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**	**-**

3. Mix the reaction mix thoroughly and dispense appropriate volumes into reaction tubes.

4. Add template DNA to the individual tubes containing the reaction mix.

5. Program the thermal cycler according to the manufacturer's instructions. Each program must start with an initial heat activation step at 95°C for 15 minutes. See table 2 for an example.
   For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.

6. Place the tubes in the thermal cycler and start the reaction.

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Table 2. Three-step PCR program

Stage 1	Pre-denaturation	1 cycle	95 °C	15 min[a]
Stage 2	Cycles	25-35 cycles	95 °C	20 - 30 sec[b]
			50 – 65 °C	20 - 40 sec[c]
			72 °C	60 sec/kb[d]
Stage 3	Final extension	1 cycle	72 °C	5 min[e]
	Hold	1 cycle	4 – 12 °C	∞

a. For activation of the Owi Taq Hot Start Polymerase.
b. Denaturation step: This step is the first regular cycling event and consists of heating the reaction to 95 °C for 20 – 30 seconds. It causes melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.
c. Annealing step: The reaction temperature is lowered to 50 – 65 °C for 20 – 40 seconds allowing annealing of the primers to the single-stranded DNA template. Typically, the annealing temperature is about 3 – 5 °C below the Tm (melting temperature) of the primers used.
d. Extension/elongation step: Owi Taq Hot Start Polymerase has its optimum activity temperature at 72 °C. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand. The extension time depends on the length of the DNA fragment to be amplified. As a rule of thumb, at its optimum temperature the DNA polymerase will polymerize a thousand bases per minute.
e. Final elongation: This single step is occasionally performed at a temperature of 72 °C for 5 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended.

NOTES:

Template

DNA template type	Input amount for 50 µL reaction mixture*
Genomic DNA (human, animals, or plants)	1 ng – 200 ng
Genomic DNA (*E. coli*, lambda genome)	1 ng – 10 ng
Plasmid DNA	0.1 ng – 1 ng

- For longer DNA targets more DNA polymerase could be added to the PCR master mix.

- The final $MgCl_2$ concentration of this Owi Taq Hot Start Master Mix Ready to Load is 1.5 mM. In some applications, more than 1.5 mM $MgCl_2$ is required for best results. Use 25 mM to adjust the Mg^{2+} concentration according to table 3.

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 $MgCl_2$ (µL)	0	1	2	3	4	5	6

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