First*Taq* 2X PCR Master Mix with Standard Buffer

Catalog: RK20622
Size: 100 RXN/500 RXN
Component:

First*Taq* 2X PCR Master Mix with Standard Buffer	RM20354

Product Description

First*Taq* 2X Master Mix with Standard Buffer is an optimized blend of *Taq* Klenow and Deep Ocean DNA Polymerases ideally suited for routine PCR applications using a variety of templates, including pure DNA solutions, bacterial colonies, and cDNA products. The 3´- 5´ exonuclease activity of Deep Ocean DNA Polymerase increases the fidelity and robust amplification of *Taq* Klenow DNA Polymerase. This convenient master mix contains dNTPs, $MgCl_2$, buffer components, and stabilizers, requiring only the addition of primers and DNA template for robust amplification.

Storage Temperature: -20°C

5' ->3' Exonuclease: No

3' ->5' Exonuclease: Yes

Resulting Ends:
Single-base 3´ Overhangs

Heat Inactivation: No

Error Rate:
< $140x10^{-6}$bases

1X Master Mix Composition:
20 mM Tris-HCl, 22 mM KCl, 22 mM NH_4Cl, 1.8 mM $MgCl_2$, 0.2 mM dNTPs, 25 units/ml First*Taq* DNA Polymerase, 0.06% IGEPAL CA-630, 0.05% Tween 20, 5% Glycerol, pH8.9 @ 25°C

Instructions

Reaction setup: We recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (94°C).

Add to a sterile thin-walled PCR tube:

Component	25 µl	50 µl	Final Conc.
Nuclease-free water	to 25 µl	to 50 µl	
10 µM Forward Primer	0.5 µl	1 µl	0.2 µM
10 µM Reverse Primer	0.5 µl	1 µl	0.2 µM
Template DNA	variable	variable	< 1,000 ng
First*Taq* 2X Master Mix with Standard Buffer	12.5 µl	25 µl	1X

Notes: Gently mix the reaction. Collect all liquid in the bottom of the tube by a quick spin if necessary. Overlay the sample with mineral oil if using a PCR machine without a heated lid.

Transfer PCR tubes to a PCR machine and begin thermocycling:

Thermocycling conditions for a routine PCR:

STEP	TEMP	TIME
Initial Denaturation	94°C	30 seconds
30 Cycles	94°C	15-30 seconds
	45-68°C	15-60 seconds
	68°C	1 minute per kb
Final Extension	68°C	5 minutes
Hold	4-10°C	

General Guidelines:

1. Template:

The use of high quality, purified DNA templates greatly enhances the success of PCR. Recommended amounts of DNA template for a 50 µl reaction are as follows:

DNA	Amount
genomic	1 ng–1 µg
plasmid or viral	1 pg–1 ng

2. Primers:

Oligonucleotide primers are generally 20–40 nucleotides in length and ideally have a GC content of 40–60%. Computer programs can be used to design or analyze primers. The final concentration of each primer in a PCR may be 0.05–1 µM, typically 0.2 µM

3. Mg++ and Additives:

Mg++ concentration of 1.5–2.0 mM is optimal for most PCR products generated with First*Taq* DNA Polymerase. The final Mg++ concentration in 1X First*Taq* Master Mix with Standard Buffer is 1.8 mM. This supports satisfactory amplification of most amplicons. However, Mg++ can be further optimized in 0.2 mM increments using $MgCl_2$.

For amplification of difficult targets, like GC-rich sequences, we recommend First*Taq* 2X Master Mix with GC Buffer. Alternatively, DMSO or formamide may be used.

4. Denaturation:

An initial denaturation of 30 seconds at 94°C is sufficient to amplify most targets from pure DNA templates. For difficult templates such as GC-rich sequences, a longer denaturation of 2–4 minutes at 94°C is recommended prior to PCR cycling to fully denature the template. Alternatively, use First*Taq* 2X Master Mix with GC Buffer. With colony PCR, an initial 2–5 minute denaturation at 94°C is recommended to lyse cells. During thermocycling, a 15–30 second denaturation at 94°C is recommended.

5. Annealing:

The annealing step is typically 15–60 seconds. Annealing temperature is based on the Tm of the primer pair and is typically 45–68°C. Annealing temperatures can be optimized by doing a temperature gradient PCR starting 5°C below the calculated T_m.

6. Extension:

The recommended extension temperature is 68°C. Extension times are generally 1 minute per kb. A final extension of 5 minutes at 68°C is recommended.

7. Cycle Number:

Generally, 25–35 cycles yield sufficient product. Up to 45 cycles may be required to detect low copy number targets.

8. PCR Product:

The majority of the PCR products generated using First*Taq* DNA Polymerase contain dA overhangs at the 3´ end; therefore, the PCR products can be ligated to dT/dU-overhang vectors.