NOYA-AMPLI 2X Master Mix

Catalog number: N-1161

« Research Use Only »

Overview

The Polymerase Chain Reaction (PCR) is a powerful and sensitive technique for DNA amplification (1). *Taq* DNA Polymerase is an enzyme widely used in PCR (2). The following guidelines are provided to ensure successful PCR using NOYA-AMPLI 2X Master Mix. These guidelines cover routine PCR. Amplification of templates with high GC content, high secondary structure, low template concentrations, or amplicons greater than 2 kb may require further optimization.

Storage:

Store at -20˚C.
Volume: 1ml

Protocol

Reaction setup:

We recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (95°C).

Component	**20µl reaction**	**Final Conc.**
10 µM Forward Primer	0.4 µl	0.2 µM (0.05–1 µM)
10 µM Reverse Primer	0.5 µl	0.2 µM (0.05–1 µM)
Template DNA	variable	<1,000 ng
AMPLI-2X Master Mix	10 µl	1X
Nuclease-free water	to 20 µl	

Notes: Gently mix the reaction. Collect all liquid to 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 from ice to a PCR machine with the block preheated to 95°C and begin thermocycling:

Thermocycling conditions for a routine PCR:

STEP	TEMP	TIME
Initial Denaturation	95°C	3 minutes
30 Cycles	95°C 45-68°C 72°C	15-30 seconds 15-60 seconds 1 minute per kb
Final Extension	72°C	5 minutes
Hold	4-10°C	

General Guidelines:

1. Template:

Use of high quality, purified DNA templates greatly enhances the success of amplification. Recommended amounts of DNA template for a 20 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 such as Primer3 (http://frodo.wi.mit.edu/primer3) can be used to design or analyze primers. The final concentration of each primer in a reaction may be 0.05–1 µM, typically 0.1–0.5 µM.

3. Mg^{++} and additives:

Mg^{++} concentration of 1.5–2.0 mM is optimal for most PCR products generated with *Taq* DNA Polymerase. The final Mg^{++} concentration in *AMPLI-2X Master Mix* is 1.5 mM. This supports satisfactory amplification of most amplicons. However, Mg^{++} can be further optimized in 0.5 or 1.0 mM increments using $MgCl_2$. Amplification of some difficult targets, like GC-rich sequences, may be improved with additives, such as DMSO (3) , formamide (4) or GC solve buffer.

4. Denaturation:

An initial denaturation of 10min at 95°C is prerequisite for activation of taq enzyme. During thermocycling a 15–30 second denaturation at 95°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 Tm. When primers with annealing temperatures above 65°C are used, a two-step PCR protocol is possible (see #8).

6. Extension:

The recommended extension temperature is 72°C. Extension times are generally 1 minute per kb. A final extension of 5 minutes at 72°C is recommended.

7. Cycle number:

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

8. Two- step PCR:

When primers with annealing temperatures above 65°C are used, a two-step thermocycling protocol is possible.

Thermocycling conditions for a routine two-step PCR:

STEP	TEMP	TIME
Initial Denaturation	95°C	30 seconds
30 Cycles	95°C 65-68°C	15-30 seconds 1 minute/kb
Final Extension	65-68°C	5 minutes
Hold	4-10°C	

PCR product:

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

References

1. Saiki R.K. et al. (1985). *Science*. 230, 1350-1354.
2. Powell, L.M. et al. (1987). *Cell*. 50, 831-840.
3. Sun, Y., Hegamyer, G. and Colburn, N. (1993). *Biotechniques*. 15, 372-374.
4. Sarkar, G., Kapelner, S. and Sommer, S.S. (1990). *Nucleic Acids Res.* 18, 7465.

CAUTION

All reagents in this kit are intended for research purposes. Do not use for diagnosis or clinical purposes. Please observe general laboratory precaution and utilize safety while using this kit.

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