Hot Start PCR Master Mix, 2x

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
AP0200201	100 rxn of 50 µl	2 x 1.25 ml Hot Start PCR Master Mix

COMPONENT	COMPOSITION
Hot Start PCR Master Mix	Optimized 2x Hot Start PCR Master Mix

*For reaction optimization. PCR Enhancer (BR1900201) for all reactions to be ordered separately.

STORAGE –20°C (until expiry date – see product label)

FEATURES

- Highest PCR sensitivity without prolonged reactivation
- Optimized PCR Master Mix for minimal hands-on and fast setup
- Exceptionally pure Hot Start *Taq* DNA Polymerase and highest quality dNTPs

APPLICATIONS

- High-specificity and high-throughput hot-start PCR up to 5 kb
- Amplification of low-copy-number targets
- TA cloning

DESCRIPTION

Apsalagen™ Hot Start PCR Master Mix is a perfect choice for a fast reaction setup that reduces the time required for calculation and pipetting and eliminates the need for buffer optimization. It is designed for low-background, high-throughput PCR of 0.2–5 kb DNA targets.

The 2x Hot Start PCR Master Mix contains pure Hot Start *Taq* DNA Polymerase, extremely high-quality dNTPs and optimized PCR buffer; thus, only template, PCR primers and PCR-grade water are added.

The Hot Start *Taq* DNA Polymerase is inactive during reaction setup due to the bound antibody, which is quickly released at elevated temperatures, ensuring the enzyme is active only during PCR. There is no need for prolonged heating or denaturation steps. The hot start minimizes primer–dimers and mispriming.

The optional use of 5× PCR Enhancer improves PCR results in many cases, including impure template or low template abundance.

PROTOCOL

Prevention of PCR contamination

When assembling the amplification reactions, care should be taken to eliminate the possibility of contamination with undesired DNA.

- Use separate clean areas for preparation of samples and reaction mixtures and for cycling.
- Wear fresh gloves. Use sterile tubes and pipette tips with aerosol filters for PCR setup.
- Use only water and reagents that are free of DNA and nucleases.
- With every PCR setup, perform a contamination control reaction that does not include template DNA.

Standard PCR setup

The standard PCR protocol using reaction buffer provides excellent results for most applications. Optimization might be necessary for certain conditions, such as the amplification of long targets, high GC or AT content, strong template secondary structures or insufficient template purity. In such cases, optimization of template purification, primer design and annealing temperature is recommended.

The best conditions for each primer-template can be optimized with the following:

- Choosing the optimal quantities of template and primers
- Using a PCR Enhancer for low amounts of template, impure or GC-rich templates
- Optimizing cycling conditions

BASIC PROTOCOL

- The Master Mix is designed to be used without any optimization as it has all necessary reaction components in optimal amounts for successful PCR.

- Optionally, use the 5× PCR Enhancer (BR1900201) to increase the yield and to lower the background in more complicated PCR reactions. Thaw on ice and mix all reagents well.
- Keep all reagents and reactions on ice.
- Pipet the master mix into thin-walled 0.2 ml PCR tubes.
- Add template and primers separately if they are not used in all reactions.

COMPONENT	VOLUME	FINAL CONCENTRATION
Hot Start PCR Master Mix, 2×	25 µl	1×
5× PCR Enhancer (optional)	10 µl	1×
* For reaction optimization. PCR Enhancer (BR1900201) for all reactions to be ordered separately.		
Forward primer	Variable	0.2–1 µM
Reverse primer	Variable	0.2–1 µM
Template DNA	Variable	10 pg–1 µg
	Use 0.01–1 ng for plasmid or phage DNA and 0.1–1 µg for genomic DNA	
Nuclease free water	Variable	
Total volume	50 µl	

- Mix and centrifuge briefly to collect the liquid in the bottom of the tube.
- Place in the PCR cycler.

CYCLING PROGRAM

STEP	TEMPERATURE	TIME	CYCLES
Initial activation	95°C	2 min	1
Denaturation	95°C	30 s	25–35
Annealing	55°C	15–30 s	25–35
	Recommended annealing temperature is 2°C above Tm of primers, or use gradient PCR to optimize the annealing temperature		
Extension	72°C	30–60 s/kb	25–35
Final extension	72°C	5 min	1
	To extend all incomplete PCR products		
Storage in the cycler	4°C	Indefinitely	1

- Add loading dye solution (see DNA Loading Dye, 6×, cat. no. BR0800301) to the reactions to analyze PCR products on a gel or store them at –20°C.

CERTIFICATE OF ANALYSIS

Quality Control

Functional assay

Human genomic DNA was amplified using the Hot Start PCR Master Mix and specific primers to produce a distinct band.

Quality confirmed by: Head of Quality Control

USEFUL HINTS

- Visit Support at www.apsalagen.com for product selection guides.
- Visit Applications at www.apsalagen.com for more nucleic acid purification and analysis products.
- Visit OEM at www.apsalagen.com for custom product formulations and bulk amounts.

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