BenTaq HotStart PCR Master Mix, lyophilised

cat#BBe001

Size: 50 x 50 µl reactions

Storage

Room temperature or below for the lyophilized Master Mix, until expiry date – see product label

-20°C for reconstituted Master Mix, up to 12 months

Features

- Ready-to-use PCR master mix. Simply add primers and template
- Lyophilized formulation enables better stability at room temperature
- Ideal for a wide range of applications including routine PCR and molecular diagnostics
- Avoids non-specific amplification
- Room temperature reaction setup

Description

BenTaq HotStart PCR Master Mix contains a highly pure HotStart *Taq* polymerase, reliable for routine and demanding PCR applications. The HotStart *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. The HotStart feature minimizes primer–dimers and mispriming. The dried format allows shipment and storage for a long time at ambient temperature.

Protocol

First step: Reconstitute the lyophilized BenTaq HotStart PCR Master Mix

- Transfer the whole content of one BUFFER BenTaq HotStart to one vial of BenTaq HotStart PCR Master Mix 2x
- Mix well – the lyophilisate will dissolve within seconds
- **Store the reconstituted BenTaq HotStart PCR Master Mix at -20°C**

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

This standard protocol 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 can be optimized with the following:

- Choosing the optimal quantities of template and primers
- Optimizing cycling conditions

- Adding betaine or DMSO (suggested 2M and 10% final concentration respectively) can help in if the template have strong secondary structures or it is of insufficient purity

Standard 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.

- Thaw the reconstituted BenTaq HotStart PCR Master Mix 2x on ice and mix 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 ratios for 50 µl PCR reaction

For total reaction volumes other than 50 µl, scale reagents proportionally.

Component	**Volume**	**Final concentration**
Reconstituted BenTaq HotStart PCR Master Mix 2x	25 µl	1×
Forward primer	Variable	0.2–1 µM
Reverse primer	Variable	0.2–1 µM
Template DNA*	Variable	10 pg–1 µg
Nuclease free water	Variable	-
Total volume	**50 µl**	

*Use 0.01–1 ng for plasmid or phage DNA and 0.05–1 µg for genomic DNA

- 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*	(55-68°C)	15–30 s	
Extension	72°C	30–60 s/kb	
Final extension	72°C	5 min	1
Storage in the cycler	4°C	Indefinitely	1

*Recommended annealing temperature is 2°C above Tm of primers.

Add the loading dye to the reactions to analyze PCR products on an agarose gel or store the completed PCR reaction at −20°C.