BenTaq HiFi PCR Master Mix, 2x

cat#BBe003

Size: 50 x 50 µl reactions

Storage

Room temperature up to 1 month
4°C up to 6 months
-20°C up to 1 year

Note: BenTaq HiFi PCR is light sensitive and should be stored and protected from light.

Features

- Ready-to-use PCR master mix. Simply add primers and template.
- Contains DNA polymerase with improved fidelity and processivity
- Effective for GC-rich targets
- Direct DNA band visualization using blue or UV light

Description

The BenTaq HiFi PCR is a pre-mixed solution containing GDP-HiFi DNA polymerase, buffer, dNTPs, enhancer, gel loading dyes, and fluorescence dye. The GDP-HiFi DNA polymerase supplied in the mixture is a new recombinant enzyme which has 70 times better fidelity and faster elongation rate (as fast as 15 seconds per kilo base, kb) than *Taq* DNA polymerase. GDP-HiFi shows higher stability at high temperature, making GDP-HiFi DNA polymerase a good choice for GC-rich templates. GDP-HiFi DNA polymerase produces blunt end PCR products. The dyes included in the BenTaq HiFi PCR mix allow instantaneous band visualization using a blue or UV light detector and provide a safe, non-toxic and non-mutagenic alternative to ethidium bromide.

Protocol

1. For each 50 µl reaction, assemble the following components in a 0.2 ml PCR tube on ice just prior use:

Component	**Volume**	**Final concentration**
BenTaq PCR HiFi 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

2. Mix gently and centrifuge briefly, close the tubes and place them in the thermal cycler.

Cycling Program

Step	**Temperature**	**Time**	**Cycles**
Initial activation	94°C	2-5 min	1
Denaturation	94°C	20-40 s	25–35
Annealing*	(55-68°C)	15–30 s	
Extension	72°C	2 mins	
Final extension	72°C	5 min	1
Storage in the cycler	4°C	Indefinitely	1

*Recommended annealing temperature is 5°C below the Tm of primers.

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

3. After the PCR reaction, use DNA electrophoresis to detect the PCR product.

Note: when the DNA concentration is less than 4 pg, the fluorescent dye may cause a migratory shift when performing electrophoresis. To avoid the shift you can remove the fluorescent dye performing the following steps:

1. Immerse the PCR product containing the fluorescence dye into the 100 mM NaCl and add 2.5 volumes of absolute or 95% ethanol.
2. Incubate on ice for 20 minutes.
3. Centrifuge the mixture at 4°C for at least 10 minutes.
4. Remove the suspension of ethanol and wash the pellet with 1ml of 70% ethanol.
5. Dry the residual ethanol and resuspend the double-stranded DNA in the TE or DNAse RNAse free double distilled water.

Caution

- During operation, always wear a lab coat, disposable gloves, and protective equipment.
- Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.