



BenTaq PCR Master Mix, 2x

cat#BBe002

Size: 50 x 50 μ l reactions

Storage

Room temperature up to 3 months

4°C up to 6 months

-20°C up to 1 year

Note: BenTaq 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 *Taq* DNA polymerase
- Direct DNA band visualization using blue or UV light

Description

The BenTaq PCR Master Mix 2x is a pre-mixed solution containing *Taq* DNA polymerase, buffer, dNTPs, gel loading dyes, and fluorescence dye. The *Taq* DNA polymerase supplied in the mixture is purified from the *E. coli* and has a 5' \rightarrow 3' DNA polymerase and the 5' \rightarrow 3' exonuclease activity but lacks the 3' \rightarrow 5' exonuclease activity. The dyes included in the BenTaq 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 μ l reaction, assemble the following components in a 0.2 ml PCR tube on ice just prior use:

Component	Volume	Final concentration
BenTaq PCR Master Mix 2x	25 μ l	1x
Forward primer	Variable	0.2–1 μ M
Reverse primer	Variable	0.2–1 μ M
Template DNA*	Variable	10 pg–1 μ g
Nuclease free water	Variable	-
Total volume	50 μl	

*Use 0.01–1 ng for plasmid or phage DNA and 0.05–1 μ g 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	30–60 s/kb	
Final extension	72°C	5 min	1
Storage in the cycler	4°C	Indefinitely	1

*Recommended annealing temperature is 5°C below the T_m 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

4. 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.