GeneDireX®

OnePCR™ HotStar

Cat. No.: MB206-0100 Size: 100 Reactions (2 X 1.25 ml)
Cat. No.: MB206-0002 Size: 2 Reactions (1 X 50 µl)

Description

OnePCR™ HotStar is a ready-to-use PCR reaction mixture. Simply add primers, template, and water, the reagent will execute primer extensions and other molecular biology applications. OnePCR™ HotStar is a pre-mixed solution containing Hot start *Taq* DNA polymerase, PCR buffer, dNTPs, gel loading dyes, enhancer, and fluorescence dye. OnePCR™ HotStar, which contains the fluorescence dye, is directly detected on BLooK LED transilluminator or UV epi-illuminator after the DNA electrophoresis. Hot start *Taq* DNA polymerase has a non-template–dependent terminal transferase activity that adds a 3' deoxyadenosine to product ends, and has a 5'→3' exonuclease activity (but not 3'→5' exonuclease activity). OnePCR™ HotStar mixture is supplied at the 2X concentration to allow 50% of the final reaction volume to be used for the addition of primer and template solutions. The enzyme s contains a proprietary antibody that blocks polymerase activity at ambient temperatures. Activity is restored after the initial denaturation step in PCR cycling at 94°C, thereby providing an automatic "hot start" for *Taq* DNA polymerase in PCR.

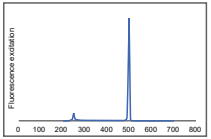

Fig. 1a. Fluorescence excitation spectra of the fluorescence dye

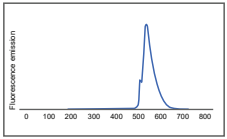

Fig. 1b Fluorescence emission spectra of the fluorescence dye

Tracking dyes

➢ Bromophenol Blue, Xylene Cyanol FF.

Features

➢ No post-staining processing of DNA required.
➢ No need to prepare PCR Reagents.
➢ Direct loading onto your agarose gel for analysis.
➢ Sensitivity – High degree of sensitivity as the ethium bromide.
➢ Speed – No destaining requirement.
➢ Compatibility – Use the Blue Light or UV to detect the signal.
➢ Specificity – Reduce the non-specific amplification products, thus significantly improving the specificity of PCR reactions.

Protocol

Standard PCR with OnePCR™ HotStar:

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

	Volume (µl)
OnePCR™ HotStar	25
Forward primer, 5~10 µM	1
Reverse primer, 5~10 µM	1
DNA template	Variable
Add ddH_2O to	50

2. Mix gently. If necessary, centrifuge briefly. Cap tubes and place in the thermal cycler.
3. Process in the thermal cycler for 25~35 cycles as follows:

Initial Denaturation	2~5 minutes at 94°C	
Denaturation	20~40 seconds at 94°C	↩ 30 cycles
Annealing	1 minute at the proper annealing temperature	
Extension	2 mins at 72°C	
Final extension	5 mins at 72°C	

Note: Optimal conditions for amplification will vary depending on the primers and thermal cycler used. It may be necessary to optimize the system for individual primers, template, and thermal cycler.

4. After the PCR reaction, DNA electrophoresis will detect the PCR product.
5. Use the UV or blue-light transilluminator or UV epi-illuminator to photograph the gel.

Note: When the DNA concentration is less than 4pg, it may cause the migratory shift when performing the electrophoresis. To remedy this observation, we recommend to conduct the following steps (please refer to the experimental procedures), or use the PCR Clean-Up & Gel Extraction Kit (see NA006-0100) to remove the flrorescence dye prior to post-staining with the Novel Green (LD002-0500) or Novel Green *plus* (LD003-0500) again for restoring the DNA molecular weight in the original position.

Removal of fluorescence dye

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.

Storage

Store at RT up to 1 month.
Store at 4°C up to 6 month.
Store at -20°C up to 1 year.
Shipping Temperature: 4°C

Note: OnePCR™ HotStar is light sensitive and should be stored and protected from light.

Related Ordering Information

Cat. No.	Description	Package
BK001	BLooK LED transilluminator	1 each
MB200-0100	PCR SuperMix	100 Reactions
MB201-0100	HotStar PCR SuperMix	100 Reactions
MB203-0100	OnePCR™	100 Reactions
MB205-0100	OnePCR™ HiFi	100 Reactions
DM101-0100	OneMARK 100	600 µl
DM110-0100	OneMARK B	600 µl

Caution:

- During operation, always wear a lab coat, disposable gloves, and protective equipment.
- All products are for research use only.