One PCR Supermix

Cat. No.: HBB203-0100 Size: 100 Reactions (2 X 1.25 ml)
Cat. No.: HBB203-0004 Size: 4 Reactions (1X 100 µl)

Description

OnePCR™ 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™ is a pre-mixed solution containing *Taq* DNA polymerase, PCR Buffer, dNTP, gel loading dyes, and fluorescence dye. OnePCR™ which contains the *Taq* DNA polymerase, is purified from the *E. coli.*, and expressing the Thermus aquaticus DNA polymerase gene. This enzyme has a 5' → 3' DNA polymerase and the 5' → 3' exonuclease activity but lacks the 3' → 5' exonuclease activity. OnePCR™, which contains the fluorescence dye, is directly detected on BLooK LED transilluminator or UV epi-illuminator after the DNA electrophoresis. OnePCR™ mixture is supplied at the 2X concentration to allow approximately 50% of the final reaction volume to be used for the addition of primer and template solutions. Reagents are provided with the sufficient amplification reactions of 50 µl each.

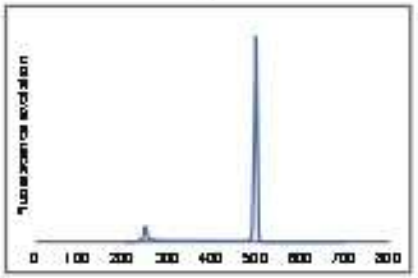

Fig. 1a. Fluorescence excitation spectra of the fluorescence dye

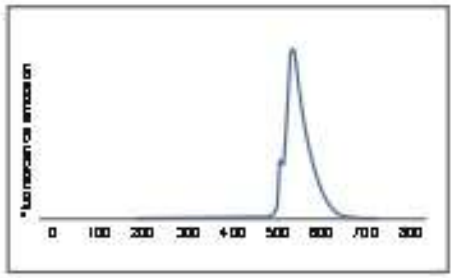

Fig. 1b Fluorescence emission spectra of the fluorescence dye

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.

Protocol

Standard PCR with OnePCR™:

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™	25
Forward primer, 5~10 µM	1
Reverse primer, 5~10 µM	1
DNA template	1
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	↰
Annealing	1 minute at the proper annealing temperature	30 cycles
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 fiorescence 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 For 3 months.
Store at 4°C For 6 months.
Store at -20°C For 1 year.
Shipping Temperature: 4°C

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

Caution:

During operation, always wear a lab coat, disposable gloves, and protective equipment.

All products are for research use only.