PCR OnePCR™ *Plus*

Cat. No.: SM207-0100 Size: 100 Reactions (2 × 1.25 ml)
Cat. No.: SM207-0004 Size: 4 Reactions (1 × 100 µl)

Description

OnePCR™ *Plus* 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™ *Plus* is a pre-mixed solution containing *Taq* DNA polymerase, PCR reaction buffers, dNTPs, gel loading dyes, and fluorescence dye. OnePCR™ *Plus* contains the *Taq* DNA polymerase, which is purified from the *E. coli.* and exhibits 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™ Plus also contains the fluorescence dye, which is directly detected on BLooK LED Transilluminator (BK001) or UV epi-illuminator after the DNA electrophoresis. OnePCR™ *Plus* 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. Reagents are provided with the sufficient amplification reactions of 50 µl each.

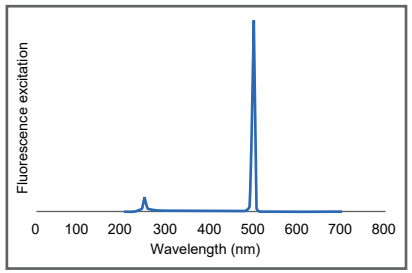

Fig. 1a. Fluorescence excitation spectra of the fluorescence dye

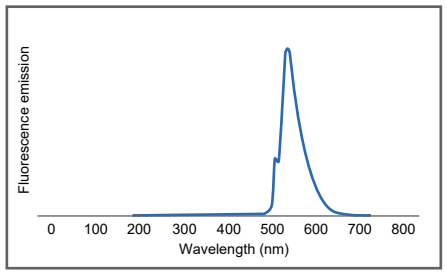

Fig. 1b Fluorescence emission spectra of the fluorescence dye

Features

- No post-staining processing of DNA required.
- No need to prepare PCR Reagents.
- Direct loading onto your agarose gel.
- Sensitivity – High degree of sensitivity as the ethidium bromide.
- Time efficiency – No destaining requirement.
- Compatibility –Use the UV light or blue light to detect the signal.

Applications

- PCR Amplification

Kit Contents

Contents	SM207-0100	SM207-0004
OnePCR™ *Plus*	1.25 ml X 2 vials	100 µl X 1 vial

Tracking Dye

- Bromophenol Blue, Xylene Cyanol FF.

Quality Control

The quality of the OnePCR™ *Plus* is tested on a lot-to-lot basis to ensure consistent product quality.

Required Materials

- Electrophoresis equipments.
- DNA Markers (optional).
- BLooK LED Transilluminator or UV epi-illuminator

Buffer Preparation

- TE buffer, pH8.0 (Selective): 10 mM Tris-HCl, pH 8.0 with 1 mM EDTA

Storage

Store at room temperature up to 3 months
Store at 4°C up to 6 months
Store at -20°C up to 1 year
Shipping temperature: 4°C

OnePCR™ *Plus* Protocol
Standard PCR with OnePCR™ *Plus*

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

Component	Volume (µl)	Final Concentration
OnePCR™ *Plus*	25	1X
Forward primer (5-10 µM)	1	0.1-0.2 µM
Reverse primer (5-10 µM)	1	0.1-0.2 µM
DNA template	Variable	-
Add ddH_2O to	50	

2. Mix gently. If necessary, centrifuge briefly. Cap the tube and place it in the thermal cycler.
3. To process in the thermal cycler for 25-35 cycles as follows:

Process	Temperature (°C)	Time	Cycles
Initial Denaturation	94	5 minutes	1
Denaturation	94	20-40 seconds	25-35
Annealing	the proper annealing temperature	1 minute	
Extension	72	2 minutes	
Final extension	72	5 minutes	1

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

4. After the PCR reaction, DNA electrophoresis will detect the PCR product.
5. Use the BLooK LED Transilluminator or UV epi-illuminator to photograph the gel.

Note: If the concentration of PCR amplification product is less than 4 pg, it may cause the migratory shift when performing the electrophoresis. To remedy this observation, we recommend to conduct the following Removal of fluorescence dye steps (please refer to the experimental procedures), or use the PCR Clean-Up & Gel Extraction Kit (catalog number: SN006-0100) to remove the fluorescence dye prior to post-staining with the Novel Green (catalog number: SL002-0500) or Novel Green *plus* (catalog number: SL003-0500) 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 buffer.

Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when you did PCR amplification with the kit.

Problem	Cause	Solution
Low yield of PCR products	Incomplete concentration of start materials	Use the appropriate method for the DNA preparation based on the amount of the starting materials.
DNA degrade	DNA is not fresh	Avoid repeated freeze / thaw cycles of the sample.
		Keep DNA preparations on ice or frozen in order to avoid the degradation.
	DNase contaminant	Use the fresh TAE or TBE electrophoresis buffer.
		Maintain a sterile work environment to avoid contamination from DNase.

Related Ordering Information

Cat. No.	Description	Size
SA001-0500	AGAROSE Tablet, 0.5g	100 Tablets
BK001	BLooK LED Transilluminator	1 Set
SD010-R600	1 Kb DNA Ladder RTU	600 µl
SN005-0100	Plasmid *mini*PREP Kit	100 Reactions

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.
- OnePCR™ *Plus* is light sensitive and should be stored and protected from light.