— PROTOCOL —

OnePCR™ Ultra

Catalog Number	Unit Size	Reactions
MB208-0100	2 × 1.25ml	100 Reactions

Storage : Store at RT up to 3 month.
Store at 4°C up to 6 month.
Store at -20°C up to 1 year.
Shipping Temperature: 4°C
Note: OnePCR™ Ultra is light sensitive and should be stored and protected from light.

Description

OnePCR™ Ultra 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™ Ultra is a pre-mixed solution containing Taq DNA polymerase, PCR buffer, dNTP, gel loading dyes, enhancer, and fluorescence dye. OnePCR™ Ultra 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™ Ultra, which contains the fluorescence dye, is directly detected on BluPAD LED transilluminator or UV epiilluminator after the DNA electrophoresis. OnePCR™ Ultra 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 ul each.

Features

- No post-staining processing of DNA required.
- Direct loading onto agarose gel for electrophoresis.
- Sensitivity – High degree of sensitivity equivalent to ethium bromide.
- Speed – Benefit for quick set up of PCR reaction such as colony PCR when screening for positive clones.
- Compatibility – Use blue light or UV to detect the signal.

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Protocol

Standard PCR with OnePCR™ Ultra:

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

	Volume (ul)
OnePCR™ Ultra	25
Forward primer, 5~10 uM	1
Reverse primer, 5~10 uM	1
DNA template	1
Add ddH2O 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 min at the proper annealing temperature
Extension	2 min at 72°C
Final extension	5 min at 72°C

30 cycles

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, run on agarose electrophoresis to detect PCR product. No additional dye is required for the PCR samples.
5. Use the UV or blue-light transilluminator or UV epiilluminator to photograph the gel.

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.

Caution:

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

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