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ProductInformation

PCR Core Kit

Product Code **CORE1**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The PCR Core Kit contains all the necessary reagents for the amplification of DNA templates by the polymerase chain reaction† with the exception of *Taq* DNA Polymerase, the DNA template and corresponding primers. All of the reagents are of very high quality and are optimized for the PCR process. This kit has been functionally tested for the amplification of a 500 base pair fragment of lambda DNA.

Components

- 10X PCR Buffer (P2192), 1 vial, 100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$ and 0.01% (w/v) gelatin
- 10X PCR Buffer II (P2317), 1 vial, 100 mM Tris-HCl, pH 8.3, 500 mM KCl
- Magnesium Chloride, 25 mM solution (M8787), 1 vial
- Water, PCR Reagent (W1754), 3 vials
- Deoxynucleotide Mix (D7295), 0.25 ml, dNTP Mix, 10 mM dATP, 10 mM dCTP, 10 mM dGTP, 10 mM TTP

Reagents and Equipment to be Supplied by User

(Product Codes are provided as appropriate.)

- *Taq* DNA Polymerase (D1806)
- Mineral Oil (M8662) (optional)
- Thermal cycler
- Primers
- DNA to be amplified
- Chloroform (C7559) (optional)
- 0.2 ml or 0.5 ml Thin-walled PCR tubes, (P3114 or P3364, respectively)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at –20 °C.

Procedure

Because *Taq* DNA Polymerase is a magnesium ion-dependent enzyme, the optimal conditions for the concentration of *Taq* DNA polymerase, template DNA, primers, and $MgCl_2$ will depend on the system being utilized. It may be necessary to determine the optimal conditions for each individual component. This is especially true for the *Taq* DNA polymerase, cycling parameters, and the $MgCl_2$ concentration. The 10X PCR Buffer II, included in this kit, contains no magnesium chloride. It is recommended the enzyme and the $MgCl_2$ be titrated to determine the optimal efficiency.

Amplification procedure

1. Add the following reagents to a 0.2 or 0.5 ml PCR microcentrifuge tube in the order given:

Volume	Reagent
__ µl	Water (for a final volume of 100 µl)
10 µl	10X PCR Buffer or 10X PCR Buffer II
__ µl	$MgCl_2$, 25 mM (if using the PCR Buffer II without $MgCl_2$)
2 µl	Deoxynucleotide Mix
__ µl	Forward primer, 0.1–1.0 µM (typically 15–30 bases in length)
__ µl	Reverse primer, 0.1–1.0 µM (typically 15–30 bases in length)
0.2–1 µl	*Taq* DNA Polymerase
__ µl	Template DNA (typically 10 ng)
100 µl	**Total volume**

2. Mix gently by vortex and briefly centrifuge to collect all components to the bottom of the tube.

3. Add 100 µl of mineral oil (optional) to the top of each tube to prevent evaporation.

4. Amplification parameters will vary depending on the primers and the thermal cycler used. It may be necessary to optimize the system for individual primers, template, and thermal cycler.

   Common cycling parameters:

   Denature the template at 94 °C for 1 minute
   Anneal primers at 55 °C for 2 minutes
   Extension at 72 °C for 3 minutes

   25–30 cycles of amplification are recommended.

5. Evaluate the amplified DNA by agarose gel electrophoresis and subsequent ethidium bromide staining. Mineral oil overlay may be removed by a single chloroform extraction (1:1), recovering the aqueous phase.

References

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† The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.

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