



Product Information

PCR Core Kit with *Taq* DNA Polymerase

Product Code **CORE-T**
Technical Bulletin MB-340

TECHNICAL BULLETIN

Product Description

The PCR[†] Core Kit contains *Taq* DNA Polymerase and all the necessary reagents for the amplification of DNA templates by the polymerase chain reaction with the exception of the DNA template and corresponding primers. All the reagents are of very high quality and are optimized for the PCR process. *Taq* DNA Polymerase is a thermostable enzyme derived from the thermophilic bacterium *Thermus aquaticus*. The enzyme is in a recombinant form, expressed in *E. coli*. It is able to withstand repeated heating to 95 °C without significant loss of activity. The enzyme is approximately 94 kDa by SDS-PAGE with no detectable contaminating endonuclease or exonuclease activity. It has 5'→3' DNA polymerase activity and 5'→3' exonuclease activity. This kit has been functionally tested for the amplification of a 500 base pair fragment of lambda DNA.

Unit Definition: One unit incorporates 10 nmol of total deoxyribonucleoside triphosphates into acid-precipitable DNA in 30 min at 74 °C.

Reagents Provided

- *Taq* DNA Polymerase, 250 units
Product Code D 6677
5 units/μl in 20 mM Tris-HCl, pH 8.0, 100 mM KCl,
0.1 mM EDTA, 1 mM DTT, 0.5% Tween 20,
0.5% Igepal CA-630, 50% glycerol
- 10× PCR Buffer, Product Code P 2192 1.5 ml
100 mM Tris-HCl, pH 8.3, 500 mM KCl,
15 mM MgCl₂, 0.01% (w/v) gelatin
- 10× PCR Buffer II, Product Code P 2317 1.5 ml
100 mM Tris-HCl, pH 8.3, 500 mM KCl
- Magnesium Chloride, Product Code M 8787 1.5 ml
25 mM MgCl₂
- Water, PCR Reagent, 1.5 ml
Product Code W 1754
- Deoxynucleotide Mix, Product No. D 7295 0.25 ml
10 mM dATP, 10 mM dCTP,
10 mM dGTP, 10 mM TTP

Materials Required But Not Provided

(Sigma product numbers provided where appropriate)

- Thermal cycler
- Dedicated pipettes
- PCR pipette tips
- 0.2 or 0.5 ml PCR microcentrifuge tubes, thin-walled, Product Codes P 3114 and P 3364
- Primers
- DNA to be amplified
- Mineral oil, Product Code M 8662 (optional)
- Chloroform, Product Code C 7559 (optional)

Precautions and Disclaimer

Sigma's Core PCR Kit containing *Taq* DNA Polymerase is for R&D. Not for drug, household or other uses. Refer to Material Safety Data Sheet.

Storage

Store all components 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 MgCl₂ 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 MgCl₂ concentration. The 10× PCR Buffer II, included in this kit, contains no magnesium chloride. It is recommended the enzyme and the MgCl₂ be titrated to determine the optimal efficiency. Sigma offers a separate PCR Optimization Kit (Product Code OPT-II) containing a variety of buffers and adjuncts for optimizing the PCR.

1. Add the following reagents to a 0.2 ml or 0.5 ml thin-walled PCR microcentrifuge tube:

Amount	Component	Final Concentration
q.s.	Water	
5 μ l	10 \times PCR Buffer or 10 \times PCR Buffer II	1 \times
- μ l	25 mM MgCl ₂ (if using 10 \times PCR Buffer II without MgCl ₂)	Typically 1.5-3.5 mM
1 μ l	Deoxynucleotide Mix, D7295	200 μ M each dNTP
- μ l	Forward Primer	0.1-0.5 μ M
- μ l	Reverse Primer	0.1-0.5 μ M
0.5 μ l	Taq DNA Polymerase	0.05 units/ μ l
- μ l	Template DNA (typically 10 ng)	200 pg/ μ l
50 μ l	Total reaction	

Note: A master mix is highly recommended when performing multiple PCR. Primers are typically 15-30 bases in length.

2. Mix gently by vortex and briefly centrifuge to collect all components to the bottom of the tube.
3. Add 50 μ l of mineral oil to the top of each tube to prevent evaporation if not using a thermal cycler with a heated lid.
4. The 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 are:

Denaturation	94 $^{\circ}$ C	1 min
Annealing	55 $^{\circ}$ C to 68 $^{\circ}$ C	2 min
Extension	72 $^{\circ}$ C	3 min

25-30 cycles of amplification are recommended.

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

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

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