

Product Information

PCR Core Kit with *Taq* DNA Polymerase

Catalog Number **CORET**

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
Catalog Number D6677
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, Catalog Number P2192 1.5 ml
100 mM Tris-HCl, pH 8.3, 500 mM KCl,
15 mM MgCl₂, 0.01% (w/v) gelatin
- 10× PCR Buffer without MgCl₂ 1.5 ml
Catalog Number P2317
100 mM Tris-HCl, pH 8.3, 500 mM KCl
- Magnesium chloride solution, 25 mM 1.5 ml
Catalog Number M8787
- Water, PCR Reagent, 1.5 ml
Catalog Number W1754
- Deoxynucleotide Mix, Catalog No. D7295 0.25 ml
10 mM dATP, 10 mM dCTP,
10 mM dGTP, 10 mM TTP

Materials Required But Not Provided

- Thermal cycler
- Dedicated pipettes
- PCR pipette tips
- 0.2 or 0.5 ml PCR microcentrifuge tubes, thin-walled, Catalog Numbers P3114 and P3364
- Primers
- DNA to be amplified
- Mineral oil, Catalog Number M8662 (optional)
- Chloroform, Catalog Number C7559 (optional)

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

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 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 10× PCR Buffer, Catalog Number P2317, included in this kit, contains no magnesium chloride. It is recommended the enzyme and the MgCl_2 be titrated to determine the optimal efficiency. Sigma offers a separate PCR Optimization Kit, Catalog Number OPT2, 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:

Volume	Component	Final Concentration
q.s.	Water, PCR Reagent	
5 μL	10× PCR Buffer or 10× PCR Buffer without MgCl_2	1 ×
- μL	25 mM MgCl_2 (if using 10× PCR Buffer without MgCl_2)	Typically 1.5-3.5 mM
1 μL	Deoxynucleotide Mix, D7295	200 μM each dNTP
- μL	Forward Primer	0.1-0.5 μM
- μL	Reverse Primer	0.1-0.5 μM
0.5 μL	<i>Taq</i> DNA Polymerase	0.05 units/ μL
- μL	Template DNA (typically 10 ng)	200 pg/ μL
50 μL	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 μL 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 °C	1 min
Annealing	55 °C to 68 °C	2 min
Extension	72 °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.

IGEPAL is a registered trademark of General Dyestuff Corp.
TWEEN is a registered trademark of Croda International PLC

AF,CR,PHC 09/09-1

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications.

Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply.

Please see reverse side of the invoice or packing slip.