# DF-Pfu DNA Polymerase

**Description** DF-*Pfu* DNA Polymerase, isolated from the hyperthermophilic archae bacteria *Pyrococcus furiosus* is a thermostable Polymerase of approximately 92 kDa. The enzyme replicates DNA at 72 °C, catalyzing the polymerization of nucleotides into duplex DNA in the 5' → 3' direction in the presence of magnesium (prefers $MgSO_4$).

Unlike *Taq* DNA Polymerase, DF-*Pfu* DNA Polymerase possesses 3' to 5' exonuclease proofreading activity that enables the Polymerase to correct nucleotide-misincorporation errors. This means that DF-*Pfu* DNA Polymerase-generated PCR fragments will exhibit the lowest error rate of any thermostable DNA polymerase, a 12-fold increase in fidelity of DNA synthesis compared with *Taq* DNA Polymerase (error rate *Pfu* 1.3 x $10^{-6}$).

**Content**

| Ref No. | S108105 | 108105 | 108125 | color |
|---|---|---|---|---|
| **DF-Pfu DNA Polymerase** | **Sample size** | **500 units** | **2500 units** | **Blue** |
| **Incomplete * Reaction Buffer (10x)** | **1.8 mL** | **1.8 mL** | **4x 1.8 mL** | **Red** |
| **Complete ** Reaction Buffer (10x)** | **1.8 mL** | **1.8 mL** | **4x 1.8 mL** | **yellow** |
| **$MgSO_4$ 100 mM** | **1.0 mL** | **1.0 mL** | **4x 1.0 mL** | **white** |
| **Datasheet** | **1** | **1** | **1** | **--** |

* 200 mM Tris-HCl (pH 8.8), 100 mM KCl, 100 mM (NH4)2SO4, 1.0 % Triton X-100, 1 mg/mL nuclease-free BSA.

** 200 mM Tris-HCl (pH 8.8), 100 mM KCl, 100 mM $(NH4)_2SO4$, 20 mM MgSO4, 1.0 % Triton X-100, 1 mg/ml nuclease-free BSA.

**Applications:** DF-*Pfu* DNA Polymerase is recommended for use in PCR and primer extension reactions that require high-fidelity synthesis. DF-*Pfu* DNA Polymerase generated PCR fragments are blunt-ended, which can be used directly for blunt end ligation. DF-Pfu DNA Polymerase from Bioron GmbH is proved to be bacterial DNA-free.

**Concentration:** 5 U/µL

**Sensitivity**: very high

**Additionally provided:** 1 tube $MgSO_4$ (100 mM)

**Recommended $MgSO_4$ concentration:** 2.0 mM – 3.5 mM

**Storage condition:** -20 °C

**BIORON GmbH – In den Rauhweiden 20 - 67354 Römerberg (Germany)**
Phone +49 6232 298 45 0 - Fax +49 6232 298 45 29
info@bioron.net www.bioron.net

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**Quality Control**

- 98% protein homogeneity in 10% SDS-PAGE
- No detectable exo-/endonuclease activities
- PCR amplification tests with different templates
- PCR amplification tests without templates as Negative Control
- "no template" test with primers complementary to a region auf 16S bacterial ribosomal genes

**Pipetting scheme**

| Components | Volume / 25 µL PCR-Reaction | Final concentration |
|---|---|---|
| 10x Reaction-Buffer | 2.5 µL | 1x |
| dNTP-Mix (10 mM each) | 0.5 µL | 0.2 mM each |
| $MgSO_4$ (100 mM) | As required | 2.0 - 3.5 mM |
| Upstream Primer | variable | 0.1 - 0.5 µM |
| Downstream Primer | variable | 0.1 - 0.5 µM |
| Template DNA | variable | 1 to 100 ng |
| DF-Pfu DNA Polymerase * | 0.2 µL | 1.0 unit |
| Sterile dest. water | Adjust to 25 µL final volume | |

* DF-*Pfu* DNA Polymerase exhibits lower than that of *Taq* DNA Polymerase extension rate (0.5 kb/ min), so 2 min extension time is recommended for every 1 kb to be amplified.

**Thermocycler protocol**

| step | time | temperature |
|---|---|---|
| initial denaturation | 2 minutes | 94 °C |
| 30 cycles: | | |
| denaturation | 10 seconds | 94 °C |
| annealing | 20 seconds | 55 - 68 °C * |
| extension | 1 minute | 72 °C |

* Usually the optimal annealing temperature is 5 °C below the melting temperature of the primers.

**Notes:**
Program the cycler according to the manufacturers instructions.
Each program should start with an initial denaturation step at 94 °C for 2 to max. 5 min.
Recommended elongation time: 2 min/ 1 kb of target.
For maximum yield and specificity, temperatures (annealing) and cycling times should be optimized for each new template target or primer pair.

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info@bioron.net www.bioron.net