**Data Sheet** (30.05.2014)

# mi-*Pfu* DNA Polymerase

## Thermostable DNA Polymerase with proofreading activity

**Source:** *Pyrococcus furiosus,* gene expressed in *E. coli*

| Cat.-No. | Size | Conc. |
|---|---|---|
| mi-E7022S | 100 units | 2,5 units/µl |

For *in vitro* use only! For research only!

### Content

1. mi-*Pfu* DNA Polymerase, 100 units (2,5 u/µl)
2. mi-*Pfu* DNA Polymerase Buffer (10x conc.)

### Recommended PCR Assay (50 µl volume)

| | |
|---|---|
| 5 µl | 10x Reaction buffer with $MgCl_2$ |
| 2-100 ng | template DNA |
| 4 µl | of dNTP Mix (2.5 mM each) |
| 2µl (=0,4µM) | of forward Primer á 10pmol/µl stock |
| 2µl (=0,4µM) | of reverse Primer á 10pmol/µl stock |
| 0,5µl | mi-*Pfu* DNA Polymerase (á 2,5u/µl) |
| Fill up to 50 µl | PCR grade $H_2O$ |

### General reaction conditions.

| | | | |
|---|---|---|---|
| 95°C | 2 min. | | (initial denaturation) |
| 95°C | 20 sec | | (denaturation) |
| 42-65°C | 30 sec | 25-35 cycles | (annealing*) |
| 72-74°C | 1-2 min. | | (extension**) |
| 72-74°C | 5 min. | | (final extension) |
| 4-8°C | indefinite to store | | |

* Annealing temperature: T(anneal) °C = Tm -5°C
** Allow approx. 30 sec for every 1 kb to be amplified

**Note:** It is critical to withhold mi-*Pfu* DNA Polymerase until the addition of dNTPs. Otherwise, the proofreading activity of the polymerase may degrade the primers, resulting in nonspecific amplification and reduced product yield. Assemble components on ice!

For thermal cycler without hot lid, overlay the reaction mix with 1-2 drops of mineral oil to prevent evaporation during thermal cycling. Centrifuge the mix in a microcentrifuge for 5 sec. Immediately place the reactions in a thermal cycler preheated to 95°C. We recommend heating the samples at 95°C for 1-2 min. to ensure that the target DNA is completely denatured. Incubation for longer than 2 min. at 95°C is unnecessary and may reduce the yield due to DNA damage.
Start the thermal cycling program. The cycling profile given above may be used as a guideline. Optimize the amplification profile for each primer/target combination.

### Description

mi-*Pfu* DNA Polymerase is the ideal choice for applications where the efficient amplification of DNA with highest fidelity is required.
The enzyme is a genetically engineered Pfu DNA polymerase, but showing a 2-fold higher accuracy and an increased processivity, resulting in shorter elongation times.
mi-*Pfu* DNA Polymerase is a thermostable enzyme isolated from *Pyrococcus furiosus*. mi-*Pfu* DNA Polymerase catalyzes the DNA-dependent polymerization of nucleotides into duplex DNA in the 5'→3' direction in the presence of magnesium ions. The enzyme also possesses a 3'→5' exonuclease (proofreading) activity. Base misinsertions that may occur during polymerization are rapidly excised by the proofreading activity. Consequently, mi-*Pfu* DNA Polymerase is useful for polymerization reactions requiring high fidelity synthesis.

### Fidelity of the enzmye

mi-*Pfu* DNA Polymerase is characterized by a 50-fold higher fidelity compared to Taq polymerase and a 2-fold higher fidelity compared to standard Pfu polymerase.
ER mi-*Pfu* DNA Polymerase = $0.25x10^{-6}$

### General Considerations

*regarding the Enzyme:* We recommend 1.25 units (0,5µl) of mi-*Pfu* DNA Polymerase per 50 µl of amplification volume. The inclusion of more enzyme will increase the chance of primer degradation due to the intrinsic 3'→5' exonuclease (proofreading) activity. Therefore it is also essential to withhold mi-*Pfu* DNA Polymerase from the reaction until after the addition of the dNTP mix by assembling the components on ice.
*regarding Extension Time:* The extension rate of mi-*Pfu* DNA Polymerase is higher than that of *Taq* DNA Polymerase. Therefore, allow approximately 30 sec for every 1 kb to be amplified during the extension step. For most reactions, 25-35 cycles are sufficient.

### Unit definition

One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nmol of dNTP into an acid-insoluble form in 30 minutes at 74°C.

**Store** at -20°C, avoid frequent thawing and freezing.