Data Sheet (22.10.2007)

mi-*Pfu* Set

Thermostable DNA Polymerase with proofreading activity

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

Cat.-No.	Size	Conc.
mi-E6022	250 units	5 units/µl

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

Content

1. mi-*Pfu* DNA Polymerase, 250 units (5 u/µl)
2. dNTP mixture, 800 µl (2.5 mM each, in water (sodium salts, pH 7-9) Purity: ≥ 98% for each dNTP)
3. 10x Buffer ($MgCl_2$ 15 mM), 1000 µl
4. 6x Loading dye, 500 µl

Recommended PCR Assay (50 µl volume)

5 µl	10x Reaction buffer with $MgCl_2$
0.25-0.5 µl	*Taq* Pol (1.25-2.5 u)
4 µl	of dNTP Mix (2.5 mM each)
0.2-1 µM	of each Primer
2-50 ng	template DNA
Fill up to 50 µl	PCR grade H_2O

General reaction conditions.

95°C 1 to 2 min.		(initial denaturation)
95°C 0.1-1 min. 42-65°C 30 sec. 72-74°C 2-4 min.	5-35 cycles	(denaturation) (annealing*) (extension**)
72-74°C 5 min. 4-8°C indefinite to store		(final extension)

* Annealing temperature: TA°C = Tm -5°C
** Allow approx. 2 min. 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 a thermostable enzyme of approximately 92 kDa 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 posesses a 3'→5' exomuclease (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.

General Considerations.

regarding the Enzyme: We recommend 1.25 units 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 Primer Design: The sequences of the primers are a major consideration in determing the optimal temperature of the PCR amplification cycles. For primers with a high Tm, it may be advantageous to increase the annealing temperature. Higher temperatures minimize nonspecific primer annealing, increase the amount of specific product and reduce the amount of primer-dimer formation. Primers can also be protected by introducing phosphorothioate bonds (PTO) at their 3' termini.
regarding Extension Time: The extension rate of mi-*Pfu* DNA Polymerase is lower than that of *Taq* DNA Polymerase. Therefore, allow approximately 2 min. 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 72°C.

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

metabion international AG – Lena-Christ-str. 44/I – 82152 Martinsried – Tel: +49 89 899 363 0 – Fax: +49 89 899 363 11

http://www.mymetabion.com - e-mail orders to: mi@mymetabion.com