Data Sheet (15.02.2016)

mi-*Taq* Set

Thermostable DNA Polymerase

Source: *Thermus aquaticus*, gene expressed in *E. coli*

Cat.-No.	Size	Conc.
mi-E6003	500 units	5 units/µl

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

Content

1. mi-*Taq* DNA Polymerase, 500 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

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.

Taq Pol in storage buffer

20 mM Tris-HCl (pH 8.0 at 25°C), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 50% Glycerol, 0.5% Tween 20 and 0.5% NP-40

Recommended PCR Assay (50 µl volume)

5 µl	10x Reaction buffer with $MgCl_2$
0.5 µl	*Taq* Pol (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.

94°C 3 to 5 min.		(initial denaturation)
94°C 30 sec. 50-55°C* 30 sec. 72°C 1-2 min.**	25-30 cycles	(denaturation) (annealing*) (extension**)
72°C 5 min. 4-8°C indefinite to store		(final extension)

* Annealing temperature: TA°C = Tm -5°C
** Amplification time: 1 sec per 60 bp approximately

Storage conditions: - 20 ± 5°C
Avoid repeated freeze/thaw cycles

Description

mi-*Taq* DNA Polymerase is a highly pure, thermostable recombinant DNA polymerase encoded by a modified gene from *Thermus aquaticus* and expressed in *E.coli*. Its recombinant nature ensures utmost purity, reproducibility and processivity. mi-*Taq* DNA Polymerase posesses a 5'→3' polymerization-dependent exonuclease activity and lacks 3'→5' exonuclease activity. The enzyme leaves a single 3'-dA nucleotide overhang that makes the products suitable for cloning by a TA vector system.

Application

routine PCR, TA cloning, RT-PCR, multiplex PCR, PCR-SSCP, PCR-RFLP and PCR-RAPD.

Performance and purity tests

- Physical Purity: mi-*Taq* DNA Polymerase is determined to be >90% pure as judged by SDS-PAGE gel with Coomassie brilliant blue staining.
- Endonuclease Assay: No nicking activity is detectable on an ethidium bromide-stained agarose gel, when 1 µg of supercoiled plasmid DNA (pUC18) is incubated with 5 units of mi-*Taq* DNA Polymerase for 8 hours at 45°C and 8 hours at 70°C in 1x reaction buffer.
- Exonuclease Assay: No exonuclease activity is detectable on an ethidium bromide-stained agarose gel, when 1 µg of lambda DNA and 1 µg lambda/*Hind* III DNA are incubated with 5 units of mi-*Taq* DNA Polymerase for 8 hours at 45°C and 8 hours at 70°C in 1x reaction buffer.
- Functional Assay: mi-*Taq* DNA Polymerase is tested for performance in the polymerase chain reaction using 2.5 units of the enzyme to amplify lambda DNA (0.5 kb, 1.0 kb and 3 kb) and a fragment of the human beta-globin gene (408 bp).