Data Sheet (31.03.2016)

mi-*Modimerase*

Thermostable DNA Polymerase with proofreading activity

Cat.-No.	Size	Conc.
mi-E9000	200 units	5 units/µl

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

Content

1. mi-Modimerase, 200 units (5 u/µl) in 50mM TrisHCl, pH8,2; 0,1 mM EDTA; 1mM DTT; 0,1% Nonidet P40; 0,1% Tween 20; 50% glycerol
2. Dilution buffer: 50 mM TrisHCl, pH8.2 ; 0,1 mM EDTA; 1mM DTT; 0,1% Nonidet P40; 0,1% Tween 20; 50% glycerol
3. Incomplete: (10X) 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
4. Complete: (10X) 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
5. MgSO4 (100 mM)

Description

mi-*Modimerase* is a thermostable enzyme, which catalyzes the DNA-dependent polymerization of nucleotides into duplex DNA in the 5'→3' direction in the presence of magnesium ions at 72°C. 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-*Modimerase* is useful for polymerization reactions requiring high fidelity synthesis. Generated PCR products are blunt-ended, which can be used directly for blunt end ligation. The extension rate is about 0.5kb/min.

General Considerations.

regarding the Enzyme: We recommend 2.5 units of mi-*Modimerase* per 50 µl of amplification volume. Overdosing the enzyme will increase the chance of primer degradation due to the intrinsic 3'→5' exonuclease (proofreading) activity. mi-*Modimerase* prefers MgSO4 to MgCl2.

regarding modified nucleotides: We recommend to use C8-Alkyne-dUTP. C8-Alkyne-dCTP incorporation is less efficient and seems to be influenced by the GC-content of the template (GC content less than 50% is preferable).

Storage conditions: - 20 ± 5°C
Avoid frequent thawing and freezing.

Recommended PCR Assay (50 µl volume)

0.5 µl	mi-*Modimerase (2,5 U)*
5 µl	10x Reaction buffer „Incomplete“
0,5 - 1 µl	MgSO4 or MgCl2 (1 mM – 2 mM final)
Or: 5 µl	10x Reaction buffer „Complete“ (2 mM MgSO4)
x µl	of dNTP (0.2mM final each)
x µl	of each Primer (0.4 µM final)
1-10 ng	template DNA
Fill up to 50 µl	PCR grade H_2O

PCR program for up to 300 bp:

96°C for 2 min	Initial denaturation
99°C for 15 s	9 cycles
56°C for 30 sec (-1°C per cycle)	9 cycles
72°C for 30 sec	9 cycles
96°C for 15 sec	30 cycles
x°C for 30 sec*	Annealing temperature, 30 cycles
72°C for 30 sec	30 cycles
72°C for 2 min	Final extension

PCR program for up to about 2500 bp:

99°C for 2 min	Initial denaturation
99°C for 45 s	9 cycles
58°C for 30 sec (-1°C per cycle)	9 cycles
72°C for 5 min	9 cycles
99°C for 45 sec	30 cycles
x°C for 30 sec*	Annealing temperature, 30 cycles
72°C for 5 min	30 cycles
72°C for 10 min	Final extension

* Annealing temperature: TA°C = Tm -5°C

Note: It is critical to withhold mi-*Modimerase* 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!
The cycling profile given above may be used as a guideline. Optimize the amplification profile for each primer/target combination.