ProFi Taq DNA Polymerase

Kit Content

Cat. No.	*ProFi Taq* DNA polymerase	10 x Reaction Buffer	Dilution Buffer	dNTP Mixture	$MgCl_2$ Solution
E-2201	250 units	1 ml (with $MgCl_2$)	1 ml	1 ml	-
E-2202	250 units	1 ml (without $MgCl_2$)	1 ml	1 ml	1 ml
E-2203	250 units	1 ml (with $MgCl_2$)	1 ml	-	-
E-2204	250 units	1 ml (without $MgCl_2$)	1 ml	-	1 ml

Specifications

ProFi Taq DNA Polymerase

Concentration	5 units/μl
5'→3' exonuclease activity	Yes
3'→5' exonuclease activity	Yes
3' A overhang	Yes
Nuclease contamination	No
Extension rate	~20-30 kb depending on template complexity

Buffer and Reagents

10 x Reaction Buffer without $MgCl_2$: Tris-HCl, KCl, pH 9.0
10 x Reaction Buffer with $MgCl_2$: Tris-HCl, KCl, 15 mM $MgCl_2$, pH 9.0
Dilution Buffer: 20 mM Tris-HCl, 0.5 mM EDTA, 1 mM DTT, 100 mM KCl, stabilizers, 50 % glycerol, pH 8.0
dNTP Mixture: 10 mM (2.5 mM each dNTP)
$MgCl_2$ Solution: 20 mM

Storage Conditions

ProFi Taq DNA Polymerase, including buffers and reagents, should be stored immediately upon receipt at –20°C. If stored in the recommended temperature, this product will be stable until the expiration date printed out on the label.

Applications

Long-range amplification from genomic DNA, high amplification efficiency and improved fidelity, excellent performance on difficult templates, amplification of low-copy targets, high yield and high sensitivity PCR

Description

ProFi Taq DNA polymerase, developed by Bioneer, is a unique recombinant *Taq* DNA polymerase that offers enhanced amplification efficiency for PCR. *ProFi Taq* DNA polymerase provides more efficient amplification and higher fidelity than conventional *Taq* DNA polymerase. This enzyme is applicable to any template DNA, and especially effective in amplifying large genomic DNA fragments up to 20 kb. *ProFi Taq* DNA polymerase provides accurate long-range amplification of standard and complex templates and amplification of low-copy target, and is highly suitable for all PCR applications.

Unit Definition

One unit is defined as the amount of enzyme that incorporates 10 nmole of dNTP into acid-insoluble material in 30 minutes at 72°C.

Quality Assurance

Nuclease activity is not detected after incubation of 1 μg of substrate DNA – supercoiled plasmid and Lambda/*Hin*d III DNA - with 5 units of *ProFi Taq* DNA Polymerase in a 50 μl reaction volume with the supplied reaction buffer for 18 hours at 37°C and 70°C.

ProFi Taq DNA Polymerase

Protocol

1. Thaw 10 x Reaction Buffer, dNTP mix, primer solutions and template DNA.
2. Prepare a reaction mixture.

Component	20 µl reaction	50 µl reaction
Template	1 – 500 ng	1 – 500 ng
Forward primer (10 pmol/µl)	0.5-2 µl	1-5 µl
Reverse primer (10 pmol/µl)	0.5-2 µl	1-5 µl
10 x Reaction Buffer	2 µl	5 µl
10 mM dNTPs (2.5 mM each)	(Variable volume) or 2 µl	(Variable volume) or 5 µl
ProFi Taq DNA polymerase (5 units/µl)	0.1 µl	0.25 µl
PCR grade water	variable	variable

3. Mix the reaction mixture thoroughly and dispense appropriate volumes into PCR tubes.
4. Add template DNA to individual PCR tubes.
5. Perform the reaction under the following conditions.

- For Standard PCR (3-step)

Step	Temperature	Time	Cycles
Pre-denaturation	95 °C	5 min	1 cycle
Denaturation	95 °C	15-20 sec	25-35 cycles
Annealing	45-65 °C	15-30 sec	
Extension	68 °C	1 min/kb	
Final extension	68 °C	Optional. Normally 3-5 min	1 cycle

Note: Optimal annealing temperature is usually set to 5°C below the melting temperature of the primers.

- For Long targets (all targets longer than 10 kb) (2 step)

Step	Temperature	Time	Cycles
Pre-denaturation	95 °C	5 min	1 cycle
Denaturation	95 °C	15-20 sec	25-35 cycles
Annealing/extension	68 °C	<1 min/kb*	
Final extension	68 °C	Optional. Normally 3~5 min	1 cycle

*Note: Annealing/extension time depends on fragment length. Use 15 min for 20 kb, 20 min for 30 kb.

6. Maintain the reaction at 4°C after the completion of amplification. It is recommended to store samples at -20°C until use. Analyze the PCR products by agarose gel electrophoresis.

Troubleshooting

Possible Cause	Recommendation
No product or low yield	
Insufficient template	Increase the amount of template used in PCR. High quality templates are essential for amplification of long targets. Check the purity of template or repeat purification of template.
Enzyme concentration is too low	If necessary, increase the amount of enzyme in 0.5 U steps.
$MgCl_2$ concentration is too low	Increase the amount of $MgCl_2$ concentration in steps.
Primer design is not optimal	Design alternative primers.
Cycle conditions are not optimal	Reduce the annealing temperature. Increase the number of cycles.
Amplification of GC-rich genes	Add 0.5-1 M Betaine or 2-8 % DMSO.
Product is multi-banded or smeared	
Annealing temperature is too low	Increase annealing temperature according to primer length.
Incorrect extension time	Adjust the time of the extension step according to the size of the expected PCR product.
Primer design is not optimal	Design alternative primers.
Problems with template	Check the concentration, storage conditions, and quality of template.
Too many cycles	Reduce the number of cycles.
Incorrect enzyme concentration	Reduce the amount of enzyme in decrements of 0.5 U.
Products in negative control experiments	
Carry-over contamination	Set up PCR reactions in an area separate from that used for PCR product analysis.

Related Products

Cat. No.	Products
E-2205	*ProFi Taq* DNA polymerase 1,000 units, 10 mM dNTPs, 10 x Reaction Buffer with $MgCl_2$
E-2206	*ProFi Taq* DNA polymerase 1,000 units, 10 mM dNTPs, 10 Reaction Buffer without $MgCl_2$, 20 mM $MgCl_2$
E-2207	*ProFi Taq* DNA polymerase 1,000 units, 10 x Reaction Buffer with $MgCl_2$
E-2208	*ProFi Taq* DNA polymerase, 10 x Reaction Buffer without $MgCl_2$, 20 mM $MgCl_2$
D-3001	10 mM dNTP Mixture (1.0 ml, 2.5 mM each dNTP)

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