Taq DNA Polymerase

Kit Content

Cat. No.	*Taq* DNA Polymerase	10 x Reaction Buffer	Dilution Buffer	dNTP Mixture	$MgCl_2$ Solution
E-2011	500 units	1 ml (with $MgCl_2$)	1 ml	1 ml	-
E-2011-1	500 units	1 ml (without $MgCl_2$)	1 ml	1 ml	1 ml
E-2011-2	500 units	1 ml (with $MgCl_2$)	1 ml	-	-
E-2011-3	500 units	1 ml (without $MgCl_2$)	1 ml	-	1 ml

Specifications

Taq DNA Polymerase	
Concentration	5 units/µl
5'→3' exonuclease activity	Yes
3'→5' exonuclease activity	No
3' A overhang	Yes
Nuclease contamination	No
Extension rate	3–10 kb/minute depending on template complexity

Buffer and Reagents

10 x Reaction Buffer without $MgCl_2$: 100 mM Tris-HCl, 400 mM KCl, pH 9.0
10 x Reaction Buffer with $MgCl_2$: 100 mM Tris-HCl, 400 mM 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

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

Routine PCR, SYBR-Green-based qPCR, dual-labeled probe based qPCR, primer extension, TA cloning, gene sequencing

Description

Taq DNA Polymerase is a thermostable DNA polymerase that catalyzes the polymerization of nucleotides into duplex DNA in the 5' → 3' direction. Bioneer's *Taq* DNA Polymerase is isolated from recombinant *E.coli* strain containing the *Taq* DNA polymerase gene from *Thermus aquaticus* YT1. It exhibits its highest activity at pH 9.0 and 72°C.

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 *Taq* DNA Polymerase in 50 µl reaction volume with the supplied reaction buffer for 18 hours at 37°C and 70°C.

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	Variable	Variable
Forward primer (10 pmole/µl)	1 - 2 µl	2.5 - 5 µl
Reverse primer (10 pmole/µl)	1 - 2 µl	2.5 - 5 µl
10X Reaction Buffer	2 µl	5 µl
10mM dNTP (2.5mM each)	(Variable volume) or 2 µl	(Variable volume) or 5 µl
Taq DNA polymerase (5 units/µl)	0.5 - 1.0 unit	0.5 - 1.0 unit
20 mM $MgCl_2$*	(Variable volume) or 2 µl	(Variable volume) or 5 µl
PCR grade water	Variable	Variable

***:** Included in E-2011-1 or E-2011-3

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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	94°C	1 min*	1 cycle
Denaturation	94°C	15-20 sec	25-35 cycles
Annealing	AT** °C	15-30 sec	
Extension	72 °C	1 min/kb	
Final extension	72 °C	Optional. Normally 3-5 min	1 cycle

   * The Pre-denaturation step can be extended up to 5 minutes for genomic DNA.
   ** Annealing temperature (approximately 5 °C below Tm of primers)
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.

Experimental Data

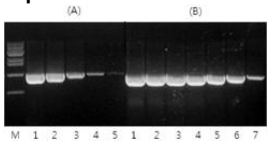

Figure 1. Bioneer's *Taq* DNA polymerase provides high amplification efficiency and sensitivity. Ten-fold serial dilutions of human genomic DNA (A) and lambda DNA (B) were amplified using 1 unit of *Taq* DNA polymerase.
Lane M: 1 kb DNA Ladder (Cat. No. D-1040, Bioneer)
Lane 1: 100 ng Template DNA
Lane 2: 10 ng Template DNA
Lane 3: 1 ng Template DNA
Lane 4: 100 pg Template DNA
Lane 5: 10 pg Template DNA
Lane 6: 1 pg Template DNA
Lane 7: 100 fg Template DNA

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-2013	*Taq* DNA Polymerase 2,000 units, 10 mM dNTPs, 10 x Reaction Buffer with $MgCl_2$
E-2013–1	*Taq* DNA Polymerase 2,000 units, 10 mM dNTPs, 10 x Reaction Buffer without $MgCl_2$, 20 mM $MgCl_2$
E-2013–2	*Taq* DNA Polymerase 2,000 units, 10 x Reaction Buffer with $MgCl_2$
E-2013–3	*Taq* DNA Polymerase 2,000 units, 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)