Top DNA Polymerase

[Cat. No.] Please refer to the **Ordering Information**

Introduction

BIONEER's *Top* DNA Polymerase is isolated from recombinant *E.coli* strain containing the DNA polymerase gene from *Thermus thermophilus*. It exhibits its highest activity at pH 9.0 and 72°C.

Applications

- Routine PCR
- Primer extension
- TA cloning
- Gene sequencing

Components

Components	E-3100	E-3100-1	E-3100-2	E-3100-3
Top DNA Polymerase	500 U (100 µl)	500 U (100 µl)	500 U (100 µl)	500 U (100 µl)
10X Reaction buffer	1 ml (with $MgCl_2$)	1 ml (without $MgCl_2$)	1 ml (with $MgCl_2$)	1 ml (without $MgCl_2$)
10 mM dNTPs	1 ml	1 ml	-	-
20 mM $MgCl_2$	-	1 ml	-	1 ml
Dilution buffer	1 ml	1 ml	1 ml	1 ml

*** Note:** For research use only. Not for use in diagnostic or therapeutic procedures.

Specifications

Top DNA Polymerase	
5' to 3' exonuclease activity	No
3' to 5' exonuclease activity	No
3'–A overhang	Yes
Fragment size	Up to 10 kb

*** Note:** This enzyme is specifically optimized for increasing base incorporation rate by inactivating 5' to 3' exonuclease activity. Therefore, this product is not recommended to use for real-time PCR using TaqMan probe.

Buffer Composition

10X Reaction buffer with $MgCl_2$	100 mM Tris-HCl, 400 mM KCl, 15 mM $MgCl_2$, etc, pH 9.0
10X Reaction buffer without $MgCl_2$	100 mM Tris-HCl, 400 mM KCl, etc, pH 9.0
Dilution buffer	50 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, stabilizer, 50% glycerol, pH 8.0

Storage Buffer

Top DNA Polymerase is supplied in 50% (v/v) glycerol containing 50 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, and stabilizer, pH 8.0.

Unit Definition

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

Quality Control

- Nuclease Contamination Assay: Nuclease activity is not detected after incubation of 1 µg of substrate DNA (supercoiled plasmid and Lambda/*Hind* III DNA) with 5 U of *Top* DNA Polymerase in 50 µl reaction volume at 37°C and 70°C for 18 hrs.

Storage

Store at -20°C. If stored in the recommended temperature, this product will be stable until the expiration date printed out on the label.

Online Resources

Korean **English**

Visit our **product page** for additional information and protocols.

Ordering Information

Description			Cat. No
500 U	10X Reaction buffer with $MgCl_2$	10 mM dNTP	E-3100
			E-3100-2
	10X Reaction buffer, 20 mM $MgCl_2$	10 mM dNTP	E-3100-1
			E-3100-3
2,000 U	10X Reaction buffer with $MgCl_2$	10 mM dNTP	E-3101
			E-3101-2
	10X Reaction buffer, 20 mM $MgCl_2$	10 mM dNTP	E-3101-1
			E-3101-3

Notice

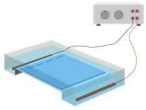
BIONEER corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

Explanation of Symbols

Experimental Procedures

<table>
<tr><th colspan="2">Steps</th><th>Procedure Details</th></tr>
<tr><td>1</td><td> 
Thaw reagents</td><td>1. Thaw 10X Reaction buffer, 10 mM dNTPs, and 20 mM $MgCl_2$ on ice and mix thoroughly before use. Then, briefly spin down all components including template DNA, Top DNA Polymerase, primers and nuclease-free water.</td></tr>
<tr><td>2</td><td> 
Preparation of reaction mixture</td><td>2. Add all components for PCR into PCR tubes (not provided) to a total volume of 20 µl or 50 µl.

• Preparation of reaction mixture
<table><tr><th>Components</th><th>20 µl reaction</th><th>50 µl reaction</th></tr><tr><td>Template DNA*</td><td>Variable</td><td>Variable</td></tr><tr><td>Forward primer (10 pmol/µl)</td><td>0.5-2 µl</td><td>1-5 µl</td></tr><tr><td>Reverse primer (10 pmol/µl)</td><td>0.5-2 µl</td><td>1-5 µl</td></tr><tr><td>10X Reaction buffer</td><td>2 µl</td><td>5 µl</td></tr><tr><td>10 mM dNTPs</td><td>2 µl</td><td>5 µl</td></tr><tr><td>20 mM $MgCl_2$†</td><td>1.5 µl</td><td>3.75 µl</td></tr><tr><td>Top DNA Polymerase (5 U/µl)</td><td>0.5-2 U</td><td>1.25-2.5 U</td></tr><tr><td>Nuclease-free water</td><td>Variable</td><td>Variable</td></tr><tr><td>Total volume</td><td>20 µl</td><td>50 µl</td></tr></table>* Recommended amounts of template DNA is as follows; plasmid and lambda DNA, > 1 pg; bacterial genomic DNA, > 10 pg; human genomic DNA, > 1 ng.
† 20 mM $MgCl_2$ is provided for E-3100-1, E-3100-3, E-3101-1, and E-3101-3.

3. Mix the reaction mixture by tapping or pipetting, and briefly spin down.</td></tr>
<tr><td>3</td><td> 
Incubate reactions in a thermal cycler</td><td>4. Perform the reaction under the following conditions.
<table><tr><th>Step</th><th>Temperature</th><th>Time</th><th>Cycles</th></tr><tr><td>Pre-denaturation</td><td>94°C</td><td>1 min</td><td>1 cycle</td></tr><tr><td>Denaturation</td><td>94°C</td><td>15-20 sec</td><td></td></tr><tr><td>Annealing</td><td>45-65°C†</td><td>15-30 sec</td><td>25-35 cycles</td></tr><tr><td>Extension</td><td>72°C</td><td>1 min/kb</td><td></td></tr><tr><td>Final extension</td><td>72°C</td><td>3-5 min</td><td>1 cycle</td></tr></table>* Note: For maximum yield and specificity, temperatures and cycling times should be optimized for each new template DNA or primers.
† Set the annealing temperature to 3-5°C lower than the Tm of the primers.</td></tr>
<tr><td>4</td><td> 
Analyze with gel electrophoresis</td><td>5. After the reaction, maintain the reaction mixture at 4-8°C. The samples can be stored at -20°C until use.

6. Load samples on agarose gel with adding a loading-dye mixture, and perform gel electrophoresis for analysis.</td></tr>
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