



For research use only

ISO9001

PyroMax DNA Polymerase

Product Description	Quantity	Cat. No.	Remarks
PyroMax DNA Polymerase	250 unit	EBT-1313	5 unit/ μ l
	500 unit	EBT-1314	5 unit/ μ l

Description

PyroMax DNA Polymerase is purified from a recombinant *E.coli* strain. This enzyme catalyzes the incorporation of nucleotides into duplex DNA in the 5'→3' direction in the presence of Mg²⁺ at 70–80°C. PyroMax DNA Polymerase exhibits 3'→5' exonuclease (proof-reading) activity, but has no detectable 5'→3' exonuclease activity.

This enzyme is designed for a reliable amplification of long, complex targets with high accuracy. Although the error rate is similar as Pfu, the amplification efficiency and extension rate is about 2 times more than Pfu. The amplified products by PyroMax DNA polymerase can be used for a cloning of your genes with decreased error rate. PyroMax DNA Polymerase, like any other polymerases showing proof-reading activity, generates a PCR products with blunt end.

PyroMax DNA Polymerase is free from any nucleic acid which can be contaminated from expression host during purification process.

PyroMax DNA Polymerase is provided with 10x optimized reaction buffer.

Storage Buffer

5 unit/ μ l in 50 mM Tris-HCl, pH8.0, 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.1% NP-40, 0.1% Tween-20, 50% Glycerol.

Unit Definition

One unit of enzyme catalyzes the incorporation of 10 nanomoles of deoxynucleotides into a polynucleotide fraction in 30min at 72°C.

10x Reaction Buffer

500 mM Tris-HCl, pH7.9, 100 mM KCl, 50 mM (NH₄)₂SO₄, 15 mM MgCl₂, 1% Triton X-100, 0.1 mg/ml BSA.

QC tests

Activity, SDS-PAGE purity, performance tests, genomic DNA contamination test, confirmation test for the absence of endo and exonucleases.

Storage Condition

Store at -20°C.

Standard Protocol

1. Prepare 50 μ l PCR solution as follows:

PCR grade distilled water :	- μ l
10x PyroMax buffer :	5 μ l
10 mM dNTP mix (2.5 mM each) :	4 μ l
Primer (10 pmol/ μ l) :	1 μ l each
Template :	0.1–10 ng
PyroMax DNA Polymerase :	0.2–0.5 μ l (1–2.5 unit)

Adjust final vol. to 50 μ l with PCR grade distilled water

*Note : Making a mixture on ice may reduce non-specific amplification
Always, PyroMax DNA Polymerase should be added last to the mixture
High concentration of enzyme or template may lead to smearing of PCR products

2. Set PCR cycling as follows :

Initial denature at 95°C : 3 min

		1–2 kbp	3–4 kbp	5–10 kbp
Denature	95°C	5 sec	10 sec	20 sec
Anneal	T _m -4°C	5 sec	10 sec	20 sec
Extend	72°C	5 sec	20 sec	10 sec/kbp

30–40 PCR cycles

Trouble-Shooting

- No products
 - Confirm your template is intact : Try another reaction with a result assured primer pair and templates
 - Be sure all the component are correctly added and working well : Sometimes low graded dNTP may inhibit the reaction, and degraded primers can result in low sized PCR fragment.
- Smear bands or smeared background
 - Reduce template concentration : High concentration of template can lead to smearing of PCR products. Generally, 1–10 ng of plasmid DNA and 10–100 ng of genomic DNA are working well
 - Reduce enzyme concentration in the reaction
 - Reduce elongation time
 - Increase annealing temperature
 - Set up a reaction mix on ice
- Non-specific bands
 - Increase annealing temperature
 - Consider using PCR additives, like 1–2% DMSO and 0.5–1x Q buffer
 - Confirm specificity of your primers
- Low yield
 - Increase enzyme concentration in the reaction
 - Increase PCR cycle number
 - Be sure appropriate concentration of your template is added