

Product Description

Robustart Taq DNA Polymerase (Glycerol-Free) is a hot-start Taq enzyme product developed by BIORI. This glycerol-free enzyme is optimized and formulated for the special requirements of lyophilized reagents. This product effectively suppresses nonspecific reactions caused by nonspecific primer annealing or primer-dimer formation during PCR setup and amplification, providing excellent specificity and more efficient amplification of low-concentration templates. It is suitable for multiplex PCR amplification and shows strong system compatibility, delivering stable amplification performance across different types of PCR reactions.

Specification

5 U/μL

Components

1. 5 U/μL Robustart Taq DNA Polymerase (Glycerol-Free)
2. 10× PCR Buffer II (Mg²⁺ free) (optional)
3. 25 mM MgCl₂ (optional)

*10× PCR Buffer II (Mg²⁺ free) does not contain dNTPs or Mg²⁺. Add dNTPs and MgCl₂ when preparing the reaction mixture.

Activity Definition

One unit (U) is defined as the amount of enzyme required to incorporate 10 nmol of deoxynucleotides into acid-insoluble material using activated salmon sperm DNA as template/primer at 74°C for 30 min.

Storage

Store at -20±5°C. Vortex before use. Avoid freeze-thaw cycles.

Quality Control

1. SDS-PAGE purity is greater than 98%.
2. Amplification sensitivity, lot-to-lot consistency, and stability are tested.
3. No exogenous nuclease activity and no contamination with exogenous endonucleases or exonucleases.

PCR Reaction Setup

Component	Volume per Reaction	Final Concentration
10× PCR Buffer II (Mg ²⁺ free) ¹	5 µL	1×
dNTPs (10 mM each dNTP)	1 µL	200 µM
25 mM MgCl ₂	2-8 µL	1-4 mM
5 U/µL Robustart Taq DNA Polymerase (Glycerol-Free)	0.25-0.5 µL	1.25-2.5 U
25× Primer Mix ²	2 µL	1×
Template	—	<1 µg/reaction
ddH ₂ O	To 50 µL	—

1. This buffer does not contain dNTPs or Mg²⁺; dNTPs and MgCl₂ must be added separately.
2. For qPCR or RT-qPCR applications, add a fluorescent probe to the reaction mixture. A final primer concentration of 0.2 µM usually gives good results. If reaction performance is suboptimal, adjust the primer concentration within 0.2-1 µM. Probe concentration can usually be optimized within 0.1-0.3 µM. Gradient experiments can be performed to determine the optimal primer and probe combination.

Reaction Conditions

Two-step Program				Three-step Program			
Step	Temperature	Time	Cycles	Step	Temperature	Time	Cycles
Hot Start	95°C	1-5 min	1	Hot Start	95°C	1-5 min	1
Denaturation	95°C	10-20 s	35-50	Denaturation	95°C	10-20 s	35-50
Annealing/Extension	56-64°C	20-60 s	35-50	Annealing	56-64°C	10-30 s	35-50
Annealing/Extension	56-64°C	20-60 s	35-50	Extension	72°C	10-60 s	35-50

Notes

1. The enzyme supports rapid hot-start activation at 95°C or 94°C for 1-5 min.
2. It has strong system compatibility and provides higher specificity and sensitivity.
3. It is suitable as a high-sensitivity PCR detection reagent and can be used for multiplex PCR amplification.
4. It has 5'-3' polymerase activity and 5'-3' exonuclease activity; it has no 3'-5' exonuclease activity and no proofreading function.
5. It is suitable for qualitative and quantitative detection by conventional PCR and RT-PCR.
6. PCR products carry an A overhang at the 3' end and can be directly used for T-vector cloning.
7. A three-step program is recommended for primers with low annealing temperatures or for amplification of fragments longer than 200 bp.