

## Product Description

Robustart Taq DNA Polymerase is a hot-start Taq enzyme product developed by BIORI. 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/ $\mu$ L

## Components

1. 5 U/ $\mu$ L Robustart Taq DNA Polymerase
2. 10 $\times$  PCR Buffer II ( $Mg^{2+}$  free) (optional)
3. 25 mM  $MgCl_2$  (optional)

\*10 $\times$  PCR Buffer II ( $Mg^{2+}$  free) does not contain dNTPs or  $Mg^{2+}$ . Add dNTPs and  $MgCl_2$  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 $^{\circ}$ C for 30 min.

## Storage

Store at -20 $\pm$ 5 $^{\circ}$ 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 $\times$ PCR Buffer II ( $Mg^{2+}$ free) <sup>1</sup> | 5 $\mu$ L           | 1 $\times$          |
| dNTPs (10 mM each dNTP)                                  | 1 $\mu$ L           | 200 $\mu$ M         |
| 25 mM $MgCl_2$                                           | 2-8 $\mu$ L         | 1-4 mM              |
| 5 U/ $\mu$ L Robustart Taq DNA Polymerase                | 0.25-0.5 $\mu$ L    | 1.25-2.5 U          |
| 25 $\times$ Primer Mix <sup>2</sup>                      | 2 $\mu$ L           | 1 $\times$          |
| Template                                                 | --                  | <1 $\mu$ g/Reaction |
| ddH <sub>2</sub> O                                       | To 50 $\mu$ L       | --                  |

1. This buffer does not contain dNTPs or  $Mg^{2+}$  ; dNTPs and  $MgCl_2$  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  $\mu$ M usually gives good results. If reaction performance is suboptimal, adjust the primer concentration within 0.2-1  $\mu$ M. Probe concentration can usually be optimized within 0.1-0.3  $\mu$ M. Gradient experiments can be performed to determine the optimal primer and probe combination.

## Reaction Conditions

| Two-step Program    |         |         |       |
|---------------------|---------|---------|-------|
| Procedure           | Temp.   | Time    | Cycle |
| Hot Start           | 95°C    | 1-5 min | 1     |
| Denaturation        | 95°C    | 10-20 s | 35-50 |
| Annealing/Extension | 56-64°C | 20-60 s | 35-50 |
| Annealing/Extension | 56-64°C | 20-60 s | 35-50 |

| Three-step Program |         |         |       |
|--------------------|---------|---------|-------|
| Procedure          | Temp.   | Time    | Cycle |
| Hot Start          | 95°C    | 1-5 min | 1     |
| Denaturation       | 95°C    | 10-20 s | 35-50 |
| Annealing          | 56-64°C | 10-30 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.