Robustart Fast Taq DNA Polymerase-V3

BiOri BIOTECH

Product Description

Robustart Fast Taq DNA Polymerase-V3 is a hot-start rapid amplification Taq enzyme product developed by our company. It is a special enzyme optimized and prepared according to the special requirements of freeze-dried reagents. This product can not only better suppresses non-specific reactions caused by primer false annealing or primer dimers during PCR system preparation and amplification, making the product highly specific and more effective for amplifying low-concentration templates, suitable for multiplex PCR amplification reactions; but also has good system adaptability, achieving stable amplification results in different types of PCR reactions.

Components

1. 5 U/μL Robustart Fast Taq DNA Polymerase-V3
2. 10×PCR Buffer Ⅱ (Mg^{2+} free) (optional)
3. 25 mM $MgCl_2$ (optional)

*10×PCR Buffer Ⅱ (Mg^{2+} free) does not contain dNTPs or Mg^{2+}. Please add dNTPs and $MgCl_2$ when preparing reaction systems.

Unit Definition

One unit of activity (U) refers to the amount of enzyme needed to incorporate 10 nmol of deoxyribonucleotides into acid-insoluble material using activated salmon sperm DNA as a template/primer within 30 minutes at 74°C.

Storage

Storage at -20±5℃. Mix thoroughly before use and avoid repeated freeze-thaw cycles.

Quality Control

1. SDS-PAGE electrophoresis purity no less than 98%
2. Amplification sensitivity, batch-batch difference, and stability.
3. No exogenous nuclease activity, no exogenous endonuclease or exonuclease contamination.

Prepare the PCR Reaction Mix

Component	Volume per Reaction	Final Concentration
10×PCR Buffer Ⅱ (Mg^{2+} free) [1]	5 μL	1×
dNTPs（10 mM each）	1 μL	200 μM
25 mM $MgCl_2$	2-8 μL	1-4 mM
5 U/μL Robustart Fast Taq DNA Polymerase-V3	0.25-0.5 μL	1.25-2.5U
25×Primer Mix [2]	2 μL	1×
Template	——	＜1 μg / Reaction
ddH_2O	To 50 μL	——

1 . This buffer does not contain dNTPs or Mg^{2+}, so they must be added to the reaction system before use.

2. If used for qPCR/qRT-PCR, a fluorescent probe needs to be added to the reaction system. Typically, a final primer concentration of 0.2 μM performs good results; if the reaction performance is poor, adjust the primer concentration within the range of 0.2-1 μM. Typically, probe concentrations are optimized in the range of 0.1-0.3 μM. Combinations of primers and probes can be tested using gradient experiments to find their optimal combination.

Thermocycling conditions

Standard PCR Program				Fast PCR Program			
Procedure	Temp.	Time	Cycle	Procedure	Temp.	Time	Cycle
Denaturation	95℃	1~5 min	1	Denaturation	95℃	30 s	1
Denaturation	95℃	10~20 s	40~50	Denaturation	95℃	1~5 s	40~45
Annealing and Elongation	56~64℃	20~60 s		Annealing and Elongation	56~64℃	5~20 s	

Notes

1. The fast DNA polymerase amplification rate is no less than 1 kb/10 s. Different PCR instruments have significant differences in their temperature ramp rates, temperature control modes, and thermal conductivity efficiencies. It is recommended to combine specific fast PCR instruments to optimize their optimal reaction conditions.
2. Strong system adaptability, higher specificity and sensitivity.
3. Suitable for use as a high-sensitivity PCR detection reagent that can be used for multiple PCR amplification reactions.
4. Has 5'-3' polymerase activity, 5'-3' exonuclease activity; no 3'-5' exonuclease activity or proofreading function.
5. Suitable for qualitative and quantitative detection of ordinary PCR and RT-PCR.
6. The PCR product has an A at the 3' end and can be directly cloned into T vectors.
7. For primers with low annealing temperatures or fragments longer than 200 bp, it is recommended to use a three-step method for amplification.