Robustart Taq DNA Polymerase

BiOri BIOTECH

Product Description

Robustart Taq DNA Polymerase is a hot-start Taq that 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 Taq DNA Polymerase
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 Taq DNA Polymerase	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

Two-step Method	Three-step Method

Procedure	Temp.	Time	Cycle	Procedure	Temp.	Time	Cycle
Denaturation	95℃	1~5 min	1	Denaturation	95℃	1~5 min	1
Denaturation	95℃	10~20 s	35~50	Denaturation	95℃	10~20 s	35~50
Annealing and Elongation	56~64℃	20~60 s		Annealing and Elongation	56~64℃	10~30 s	
				Extension	72℃	10~60 s	

Notes

1. Can be quickly hot-started at 95°C or 94°C for 1-5 minutes.
2. The system has strong adaptability and higher specificity and sensitivity.
3. It is suitable for high-sensitivity PCR detection reagents and can be used in multiplex PCR amplification reactions.
4. Has both 5'-3' polymerase and exonuclease activity; no 3'-5' exonuclease activity or proofreading function.
5. Suitable for qualitative/quantitative detection in regular PCR and RT-PCR.
6. PCR products have an A-tail at the end, which can be directly cloned into T vector.
7. For primers with low annealing temperature or amplification of fragments longer than200 bp,it is recommended to use a three-step method.