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Enseignement et Recherche

FICHE TECHNIQUE :

Taq Polymerase HOT START

Ref : PW02

Concentration : 2.5 units/µ1 **Storage:** at -20°C for two years
Quantity : 250 Units

Description

Taq Polymerase *HOT START* is a novel hot-start enzyme containing Taq polymerase and two proprietary DNA binding proteins. At room temperature, one binding protein binds to double-strand DNA template and another binding protein binds to primer. These unique formulations effectively neutralize the DNA polymerase activity at room temperature. Blocking proteins are released from templates and primers during the initial denaturation. This double blocking method has higher efficiency than antibody based, or chemically modified hot start PCR.

Highlights

- ***Taq*** Polymerase *HOT START* offers 18-fold fidelity as compared to *Taq* Polymerase.
- Extension rate is about 1-2 kb/min.
- Template-independent "A" can be generated at the 3' end of the PCR product. PCR products can be directly cloned into pEASY-T vectors.
- Reduced nonspecific amplification and primer dimer formation.
- Different from Taq antibody, no risk of contamination from mammalian DNA.
- Different from chemical modification, long denaturing step is not needed.
- Amplification of genomic DNA fragment up to 15 kb.

Applications

- Complex templates
- GC/AT-rich templates
- Multiplex PCR
- High yield PCR

Unit Definition

One unit of ***Taq*** Polymerase *HOT START* incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 minutes at 74℃.

Quality Control

Taq Polymerase *HOT START* has passed the following quality control assays: functional absence of double- and single-strand endonuclease activity; >99% homogeneous measured by SDS-PAGE. Each batch of ***Taq*** Polymerase *HOT START* has been assayed for amplification efficiency to amplify p53 gene from 10 ng of human genomic DNA.

Storage Buffer

20 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 1 mM DTT, 100 mM KCl, 50% glycerol, stabilizers

Téléphone : 041 40 42 27 / 06 59 19 60 33
Facebook/Instagram : Wiragen Academy
Site : www.wiragen.com
E-mail : contact@wiragen.com / wiragen.academy@gmail.com
Siège social : Local 01 lotissement Si Redouane 02 N°16 Senia
Siège formation : 12 Rue de Nancy, Résidence Col.Othmane, Oran

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GC Enhancer

For better amplification of GC/At-rich or complex templates, we recommend adding GC enhancer into PCR reaction. GC enhancer is provided at 10x concentration and can be used at 0.5x-5x concentration.

Kit Contents

Component	WT1-003
Taq Polymerase *HOT START*	250 U
10× ***Taq*** Buffer	1.2 ml
2.5 mM High Pure dNTPs	800 µl
10x GC Enhancer	200 µl
6x DNA Loading Buffer	500 µl

Reaction Components

Component	Volume	Final Concentration
Template DNA	Variable	As required
Forward Primer (10 µM)	1 µl	0.2 µM
Reverse Primer (10 µM)	1 µl	0.2 µM
10× ***Taq*** Buffer	5 µl	1x
2.5 mM High Pure dNTPs	4 µl	0.2 mM
Taq Polymerase *HOT START*	0.5-1 µl	1.25x2.5 units
Nuclease-free Water	Variable	-
Total volume	50 µl	-

Thermal cycling conditions

94°C	2-5min	
94°C	30 sec	30-35 cycles
50-60°C	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	

Notes

- A final concentration of 2 mM $MgSO_4$ is sufficient for most targets amplification. For some targets, more Mg^{2+} may be required.
- For optimal results, we recommend to use the 100 mM $MgSO_4$ stock to prepare a titration from 2 mM to 4 mM (final concentration) in 0.25 mM increments.
- 0.5 µl (2.5 units) enzyme is enough for per 50 µl reaction. For better amplification, up to 1 µl (5 units) enzyme can be used.
- For amplification of GC/AT-rich templates and complex templates, we suggest to use GC Enhancer.