Epitech HS Taq DNA Polymerase

FP1508941

Storage -20℃. Avoid freeze/thaw cycle.
Shipping Ultra-low temperature transportation.

Introduction

Epitech HS Taq DNA Polymerase is a chemically modified thermostable Taq DNA polymerase. It is inactive at room temperature because chemical modification groups bind to the Taq enzyme before high-temperature heating, thereby inhibiting the polymerase activity and avoiding non-specific amplification of primer extension or the formation of primer dimers. After heat shock at 95℃ for 10 minutes, the enzyme can recover its activity. In addition, Epitech HS Taq DNA Polymerase has no detectable 3'→5' proofreading exonuclease activity, but it has 5'→3' exonuclease activity and can be used for fluorescent quantitative PCR detection.

With an optimized buffer system, it is suitable for PCR reactions of DNA samples treated with bisulfite conversion. It has better amplification efficiency and sensitivity for template DNA containing uracil after bisulfite conversion. Compared with ordinary DNA, DNA after bisulfite conversion is severely damaged, which will affect the performance of PCR reactions. Similarly, for amplifying damaged DNA samples, cytosine deamination occurs spontaneously over a long period of time, and the deamination rate accelerates when the temperature rises, leading to the accumulation of uracil in DNA and free nucleotides. When other correction enzymes are ineffective, Epitech HS Taq DNA Polymerase can efficiently amplify such damaged DNA templates containing uracil.

Component List

FP1508941	Component	250U	1KU	5KU	Storage
FP1508941A	Epitech HS Taq DNA Polymerase (5U/μL)	50μl	200μl	1ml	-20℃. Avoid freeze/thaw cycle.
FP1508941B	5×Epitech HS Taq DNA Polymerase Buffer	1ml	4ml	20ml	-20℃. Avoid freeze/thaw cycle

Scope of Application

Suitable for PCR reactions of DNA samples treated with bisulfite conversion.

Precautions

In this experiment, the pretreatment process of bisulfite will directly affect the quality of template DNA; the treated template cannot be stored for a long time and should not be kept at -20℃ for more than one month.

Other Precautions

1. Excessive template DNA is prone to cause non-specific PCR products.
2. Recommendations for the use of templates: Take mammalian and E. coli genomic DNA as examples:

Reagent Name	Addition Amount
Mammalian Genomic DNA	0.1-1 μg
E. coli Genomic DNA	10-100 ng

Usage Method

Preparation before reaction system preparation:

1. Thaw and mix all solutions required for the reaction at room temperature or 4℃. Then place them on an ice bath or in an ice box. It is recommended to aliquot the reaction solutions for use to avoid repeated freeze-thaw cycles.
2. Set up the fluorescent quantitative reaction system with reference to the following table. It is recommended to prepare the fluorescent quantitative reaction system on an ice bath or in an ice box:

Component	Addition Volume per Reaction	Final Concentration
5×Epitech HS Taq DNA Polymerase Buffer	10 μL	1×
Epitech HS Taq DNA Polymerase	0.5 μL	0.05 U/μL
dNTPs	0.5 μL	0.25 mM
Primer-probe Mix	X μL	——
Template	X μL	——
ddH_2O	To 50 μL	——
Final Volume	50 μL	——

Note: a. The amounts of primers, probes, and templates can be adjusted according to actual needs.

3. Gently pipette to mix or vortex slightly, then centrifuge at room temperature for a few seconds to collect the liquid at the bottom of the tube.
4. Place each prepared PCR reaction tube in a PCR instrument and start the PCR reaction.

Reaction Procedure

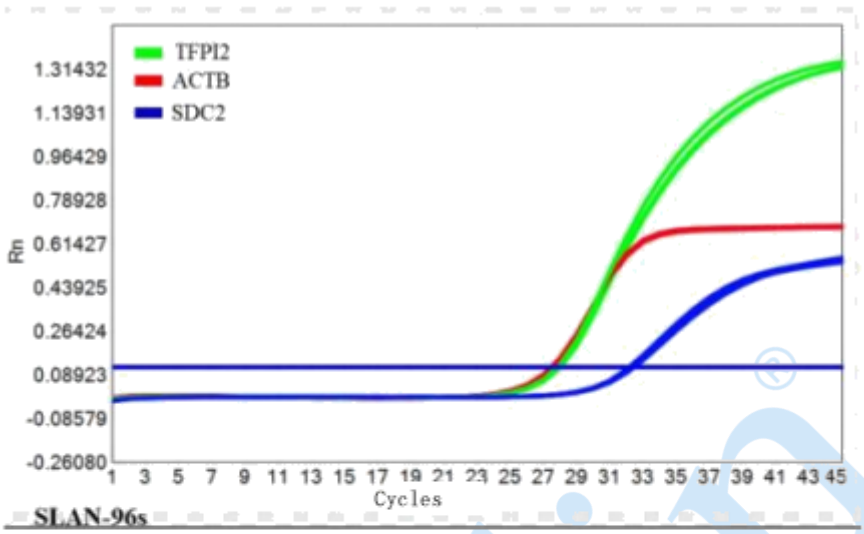
Fluorescent Quantitative PCR

Step Name	Temperature	Time	Cycles
Pre-denaturation	95 ℃	10 min	1
Denaturation[a]	94 ℃	15 s	40-45
Annealing + Extension[a]	60 ℃	40 s (Collect Fluorescence)	——

a. Reaction conditions can be adjusted according to the primers, probes, and templates.

Experimental Example

In this experiment, the colorectal cancer-related genes SDC2 and TFPI2 were used as target genes for detection, and ACTB was used as an internal reference gene. The detection results are as follows (template concentration: 10 ng/μL):



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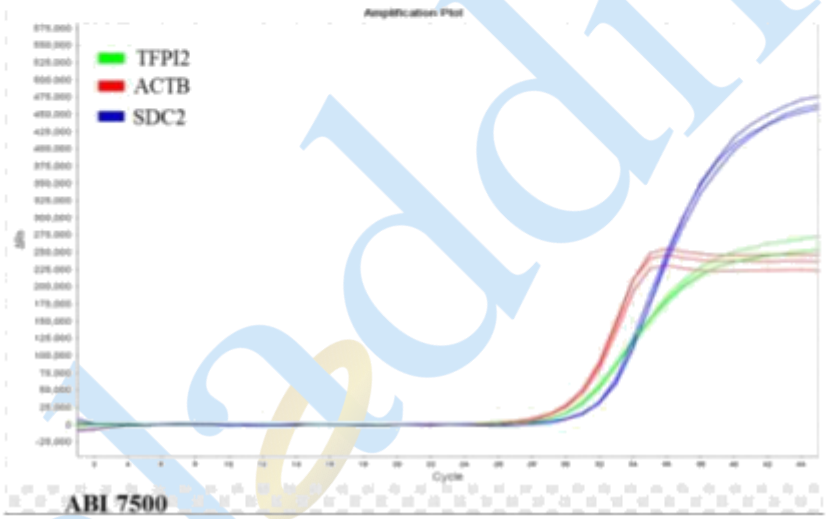

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