Tinzyme Co., Limited

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HotStart Taq DNA Polymerase

Product Number: PC17

Shipping and Storage

-20℃.

Components

Component	PC17
HotStart Taq DNA Polymerase (5U/μl)	50μl
5×HotStart Taq Buffer with Mg^{2+}	1ml×2
dNTPs (10mM each)	200μl

Description

This product is a product of Taq enzyme processed by special processes. Before heating to high temperature, the polymerase activity of Taq enzyme is inhibited, thereby inhibiting non-specific amplification caused by non-specific annealing of primers or primer dimerization under low temperature conditions (Figure 1). This product is suitable for high-specificity PCR reactions, Multiplex PCR, high GC content (>60%), secondary structure, and other genome amplification and large-scale genome amplification detection with strong background.

Features

1. Convenience: Consistent with the most effective hot start enzyme reaction conditions currently in use, there is no need to change the PCR program.
2. Efficient: Effectively reduces the generation of impurities and drags, thereby achieving high specificity PCR.
3. Sensitivity: Specific gene fragments can be amplified from a 0.05ng human genome DNA template.

Application

1. High specificity PCR reaction;
2. Complex template amplification;
3. Multiplex PCR;
4. Genomic amplification testing;
5. Fluorescence quantitative PCR.

Unit definition

After thermal activation, the amount of enzyme required to catalyze the incorporation of 10nmol dNTPs into acid insoluble substances within 30 minutes at 74℃ is one unit.

Quality control

After multiple column purification, only a clear and single target band was visible in SDS-PAGE gel detection. qPCR method detected no residual Escherichia coli DNA and no contamination of nucleic acid endonucleases and exonucleases.

Suggestion

The PCR product amplified using this reagent has a prominent "A" base at the 3 'end, which can be directly cloned into a T vector.

Protocol

1. Common reaction systems (50μl)

5×HotStart Taq Buffer with Mg^{2+*}	10μl
Upstream primer	0.2-1.0μM(Final Conc.)
Downstream primer	0.2-1.0μM(Final Conc.)
dNTPs (10mM each)	1μl
Template Taq	1-50ng(Plasmid) 10ng-1μg(Genome)
HotStart Taq DNA Polymerase	0.25μl(1.25U)
ddH_2O	Up to 50μl

Note:*The final concentration of $Mg^{2+}$is 2mM

2. Common PCR cycles

2.1. When the amplified fragment is less than 3K:

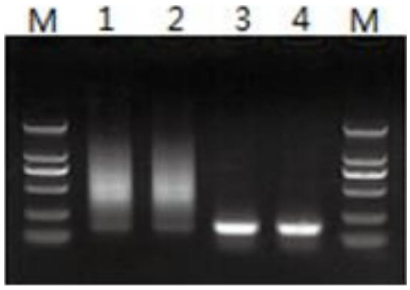
Cycle	Temperature	Time
1	94℃	300s
30	94℃	20s
	50-60℃	20s
	72℃	1kb/60s
1	72℃	5min
1	4℃	Heat preservation

2.2. When the amplified fragment is ≥ 3K (recommended primer length ≥ 30bp):

Cycle	Temperature	Time
1	94℃	5min
30	94℃	5s
	68℃	1kb/60s
1	72℃	5min
1	4℃	Heat preservation

Application instance

Figure 1) In the 50μl amplification system, a specific gene fragment (170bp) is amplified with high specificity using 50ng human genomic DNA as a template.



Swimming lane M: DNA Ladder 2000;
Swimming lane M: Taq enzyme 1.25U;
Swimming lane M: HotStart Taq enzyme 1.25U.

Note

1. 2mM Mg^{2+} can meet the vast majority of PCR amplification, and for some PCR, it can be adjusted to 2-4mM to ensure better amplification.
2. Adding the recommended enzyme amount to the system can meet the needs of most PCR amplifications. For certain PCR cases, in order to achieve better amplification, the enzyme amount can be appropriately increased.