



## Hot Exo- DNA Polymerase

Product Number: PC608

### Shipping and Storage

-20°C.

### Components

Components	PC608	PC608
	200U	1000U
Hot Exo- DNA Polymerase 2U/μl	100μl	500μl
10×Hot Exo- DNA Polymerase Reaction Buffer	1ml	4×1 ml

### Description

Hot Exo- DNA Polymerase purified self weight group E. Coli strain, this enzyme is a natural enzyme obtained through genetic engineering modification. This polymerase is a high-fidelity heat-resistant DNA polymerase with a half-life of 8 hours at 100°C. Suitable for primer extension and high-temperature (72°C) DNA sequencing.

### Unit Definition

The amount of enzyme required for the incorporation of 10 nmol of whole nucleotides into acid insoluble precipitates within 30 minutes at 75°C is defined as 1 active unit (U).

### Quality control

The purity of Hot Exo- DNA Polymerase SDS-PAGE is greater than 99%, and there is no activity of endonuclease or exonuclease.

### Protocol

Hot Exo- DNA Polymerase PCR Example:

1. Refer to the following table to set up the reaction system

Reagent	50μl Reaction system	Final Conc.
10×Hot Exo- DNA Polymerase Reaction Buffer	5μl	1×
dNTP Solution Mix (10 mM)	1μl	200μM
Upstream Primer (10 μM stock)	0.5-2.5μL	0.1-0.5μM
Downstream Primer (10 μM stock)	0.5-2.5μl	0.1-0.5μM
DNA Template	x	
Hot Exo- DNA Polymerase	0.25-0.5μl	0.5-1U
MgSO <sub>4</sub>	optional	1-6 mM
Nuclease-free water	To 50μl	

2. After setting the reaction system according to the above table, gently mix and centrifuge to precipitate the liquid.
3. Refer to the following sequencing settings for PCR sequencing.

Step	Temperature	Time	Cycles
Pre Denaturation	95°C	2-5 min	
Denaturation	95°C	15-30s	} 20-30
Annealing	55-65°C	15-30s	
Extend	72°C	1 min	
Final Extension	72°C	5 min	



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Note:1)In general experiments, the annealing temperature is 5°C lower than the melting temperature  $T_m$  of the amplification primer, and when ideal amplification efficiency cannot be achieved, the annealing temperature should be appropriately reduced; When a non-specific reaction occurs, increase the annealing temperature to optimize the reaction conditions.

2)The extension time should be set based on the size of the amplified fragment, and the amplification efficiency of Hot Exo- DNA Polymerase is 1 kb/min.

3)The number of cycles can be set based on the downstream application of the amplification product. If the number of cycles is too small, the amplification amount is insufficient; If there are too many cycles, the probability of mismatch will increase, and the non-specific background will be severe. So, while ensuring product yield, the number of cycles should be minimized as much as possible.