Hot Start Taq DNA Polymerase

NB-88-00055-100ul
NB-88-00055-500ul

Hot start Taq DNA Polymerase

#Cat: NB-88-00055-100ul Size: 100µl/250U
#Cat: NB-88-00055-500ul Size: 500µl/1250U

Cat. No	Specification	Storage/Shelf life
NB-88-00055-100ul	**100 µl/500U**	-20C°/2 years
NB-88-00055-500ul	**500 µl/2500U**	-20C°/2 years

Introduction

Hot Start Taq DNA Polymerase is a mixture of Taq enzymes incubated with Taq antibodies. Taq enzyme antibody has a very high affinity with Taq enzyme. It can block the activity of Taq enzyme before high temperature denaturation, so it caninhibit primer dimer and non-specific amplification very effectively, greatly improving the accuracy of PCR reaction.

Hot Start Taq DNA Polymerase does not require special high temperature treatment.The pre-denaturation step in conventional PCR reaction conditions can start the activity of Taq enzyme. It is suitable for various Taq enzyme-based hot-start PCR andqPCR reactions. The PCR product has an A at the 3'end, which can be cloned directly with TA vector.

Kit Components

Components	NB-88-00055-100ul	NB-88-00055-500ul
HotStart Taq DNA Polymerase (5U/µl)	**100 µl**	**500 µl**
10 × PCR Buffer (Mg2+)	**1 ml**	**5 ml**
ddH2O	**1 ml ×2**	**10ml**
User Manual	**1 copy**	**1 copy**

Unit definition

At 74°C for 30 min, the amount of enzyme required to incorporate 10 nm dNTPs into theacid-insolubleprecipitate is defined as 1 activity unit.

Activity detection conditions: 50 mM Tris-Hcl (pH 9.0, 25°C), 50 mM NaCl, 5 mM MgCl2, 0.2 mM eachdNTPs (including [3H]-Dttp), 200 µg/ml activated calf thymus DNA and 0.1 mg/mlBSA.

Quality Control

The purity detected by SDS-PAGE is greater than 99%. After detection of no exogenous nuclease activity, PCR method detects no host DNA residues, which can effectively amplify single-copy genes in the human genome.

PCR system components

1. Purity of template DNA: Many residual nucleic acid extraction reagents will affect the PCRreaction, including protease, protein denaturant (such as SDS, guanidine salt), high concentration salt (KAc, NaAc, sodium caprylate, etc.) and high concentration EDTA. The amount of template with low purity (such as the template obtained by boiling method) should not exceed 1/10 of the PCR reaction system (for example, the volume of template added to the 50 µl reaction system should not exceed 5 µl). If the purity of the template DNAis too poor, you can use our PCR product recovery kit (Cat. No. EP005) to purify and concentrate the template DNA. The amount of template purified by our PCR product recovery kit can be as much as 1/2 of the volume of the PCR reaction system.
2. The amount of template DNA: a very small amount of DNA can also be used as a template for PCR, but to ensure the stability of the reaction, it is recommended to use target sequences with more than 104 copies as a template for a 50µl system. Recommended amount of template DNA:

Human genomic DNA	0.05 µg~0.5 µg/50 µl PCR reactionsystem
Escherichia coli genomic DNA	10 ng~100 ng/50 µl PCR reaction system
λ DNA	0.5 ng~5 ng/50 µl PCR reaction system
Plasmid DNA	0.1ng ~ 10 ng/50 µl PCR reaction system

If you need to use the amplified product as a template for re-amplification, you should dilute the amplified product by at least 1,000 to 10,000 times before using it as a template, otherwise smeared bands or non-specific bands may appear.

3. Primer concentration: Generally, the concentration of each primer is 10 µM (50×), and the working concentration is 0.2 µM. Excessive primers may cause non-specific amplification, and too few primers may reduce amplification efficiency.

PCR parameter settings

1. Pre-denaturation: Generally, the pre-denaturation is 94℃, 1~5 min. Too high denaturation temperature or too long time will lose the activity of Taq enzyme.
2. Annealing: Annealing temperature is the key to PCR. Too high temperature may reduce yield, and too low temperature may produce primer dimers or non-specific amplification. It is recommended to try 5℃ lower than Tm for the first time PCR amplification (if the two primers

Tm are different, refer to the lower Tm) as the annealing temperature. Generally, the primer synthesis company will provide the Tm of the synthesized primer, and the primerTm can also be estimated according to this formula: Tm = 2℃×(A+T) + 4℃× (G+C). The optimal annealing temperature needs to be determined by gradient PCR.

3. Extension: The extension temperature is usually 72°C, and the extension time depends on the length of the target DNA fragment. The required extension time is calculated at 1kb/minTime, too long may cause non-specific increase. After the cycle is over, continue to extend for 5-10 minutes to obtain a complete double-stranded product.

4.

1. Number of cycles: 25~35 cycles are generally used, and the number of cycles can be appropriately increased for low-copy templates. However, too many cycles may increasenon-specific amplification, but not specific products.

Instructions

2. Thaw 10×PCR Buffer (Include dNTPs), ddH2O, template DNA and primers at room temperature and place on ice. 2. Turn the thawed components upside down and mix them evenly, and add them to each group in sequence as shown in the table below to make a PCR reaction system:

Components	Volume （μL)
10×PCR Buffer*	5
primer1 （10 μM）	1
primer2 （10 μM）	1
Hotstart Taq DNA Polymerase*	0.5
Template DNA	n
ddH2O	up to 50

1) 10×PCR Buffer must be thoroughly mixed before use, otherwise it will affect the PCReffect.

2) Final concentration of Mg2+: The final concentration of the system was 2mM, which satisfied the amplification of most gene fragments. If necessary, 25mM $MgCl_2$ can be used to adjust the final concentration between 2-5mM.

3) Polymerase concentration: 0.5ul/50ul system is recommended, and can be optimizedbetween 0.5-1.0ul/50uL.

The above examples are the components added in a 50 μl reaction system. If you need a reaction system with other volumes, please increase or decrease the components in proportion.

3.Flick the PCR reaction tube with your fingers to mix thoroughly, and centrifuge at lowspeed for a few seconds to allow the solution to settle to the bottom of the tube.

4. PCR reaction cycle setting example

Cycle steps	Temperature (℃)	Time	Cycle number
Pre-denaturation	94	1-5 min	1x
Denaturation	94	15 sec	25-35x
Annealing*	50-60	30 sec	
Extended※	72	30 sec	
Final extended	72	5 min	1x

*Subject to the actual best annealing temperature.

※The extension time can be designed according to the length of the amplified product, with 1kb/min as a reference.

5.Result detection: Take 5-10 µl of the amplified product directly for agarose electrophoresisdetection.

* The relationship between the concentration of agarose gel and the best resolution range of linearDNA:

Agarose concentration	Optimal range of linear DNA resolution
0.5%	1,000~30,000
0.7%	800~12,000
1.0%	500~10,000
1.2%	400~7,000
1.5%	200~3,000
2.0%	50~2,000