Pfu DNA Polymerase Ultra Pure, 5 units/ul

T2018

Storage

Store at -20°C in a constant temperature freezer.

Contents

- Product Manual
- Pfu DNA Polymerase Ultra Pure, 5 units/ul
- 5X Pfu Reaction Buffer including $MgCl_2$

Shipping Condition

Ship with dry ice.

Introduction

Pfu DNA Polymerase Ultra Pure is a Pyrococcus-like enzyme reengineered with a processivity-enhancing performance,increases fidelity and speed. Pfu DNA Polymerase Ultra Pure is an ideal choice for cloning and can be used for long or difficult amplicons. With an error rate > 50-fold lower than that of Taq DNA Polymerase and 6-fold lower than that of Pyrococcus furiosus DNA Polymerase, Pfu DNA Polymerase Ultra Pure possesses 5′→ 3′ polymerase activity, 3′→ 5′ exonuclease activity and will generate blunt-ended products.

Pfu DNA Polymerase Ultra Pure is supplied with standard 5X Pfu Reaction Buffer which contains $MgCl_2$ (1.5 mM at the final reaction concentration). Reactions can also be optimized using the Betaine, DMSO or $MgCl_2$ solutions.

Storage

20 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 200 µg/ml BSA 50% Glycerol , pH 7.4 @ 25°C.

Unit Assay Conditions

25 mM TAPS-HCl (pH 9.3 @ 25°C), 50 mM KCl, 2 mM $MgCl_2$, 1 mM b-mercaptoethanol, 200 µM dNTPs including [3H]-dTTP and 15 nM primed M13 DNA.

Quality Control Assays - Functional Assay

Pfu DNA Polymerase is tested for performance in the polymerase chain reaction (PCR) using PCR master mix,(1X)to amplify a 470bp region of the beta-globin gene human genomic DNA. The resulting PCR product is visualized on a Safe-Pinky stained agarose gel.

Reaction Setup

We recommend to assemble all reaction components on ice and quickly transfer the reactions to a thermocycler preheated to the denaturation temperature (98°C)

Components	Volume	50 ul reaction
5X Pfu Reaction Buffer	10 ul	1X
10mM dNTPS	1 ul	200 uM
Forward Primer, 10uM	2.5ul	0.5 uM
Reverse Primer, 10uM	2.5ul	0.5 uM
DNA template	1 - 5ul	< 250 ng
DMSO (Optional)	(0.1-1.5 ul)	0.2 -3%
Pfu DNA Polymerase Ultra Pure	0.2 - 0.5 ul	1- 2.5 units
Water, PCR certified	up to 50 ul	N.A

Note: For multiple reactions with common components, prepare a master mix of the commom components for all reactions to reduce pipetting errors.

Note : It is important to add Pfu DNA Polymerase Ultra Pure last in order to prevent any primer degradation caused by the 3′→ 5′ exonuclease activity.

Note : Pfu DNA Polymerase Ultra Pure may be diluted in 1X Pfu Reaciton Buffer just prior to use in order to reduce pipetting errors.

PCR Reaction Thermocycling

Step	Temp	Time	Cycle
Initial denaturation	98 °C	30 sec	1
Denaturation	98 °C	5-10 sec	25 - 35
Annealing	Primer T_M -5 °C	10-30 sec	
Extension	72 °C	15-30 sec/kb	
Final extension	72 °C	5-10 mins	1
Hold	4 °C		

Note: An initial denaturation of 30 seconds at 98 °C is sufficient for most amp -licons from pure DNA templates. Longer initial denaturation times can be used (up to 3 minutes) for templates that require it.

Note: the protocol is suggested as a strating point and guideline when using Pfu DNA Polymerase Ultra Pure. Optimal reaction conditions, such as incubation times, temperatures, and amount of template DNA, may very and must be individually determined. We recommended assembling reaction on ice from pre-chilled components.

GenDEPOT PO Box 454 Barker, Tx 77413 tech@gendepot.com

Pfu DNA Polymerase Ultra Pure, 5 units/ul

T2018

Tips

Template DNA

Usually the template DNA amount is in the range of 0.02-2ng for plasmid or phage DNA and 0.2-2µg for genomic DNA, for a total reaction mixture of 100µl. Higher template DNA amounts usually increase the yield of nonspecific PCR products,but if the fidelity of synthesis is crucial, maximal allowable template DNA quantities in action with limiting number of PCR cycles should be used to increase the percentage of "correct" PCR products. Nearly all routine methods are suitable for template DNA purification. Although even trace amounts of agents used in DNA purification procedures (phenol, EDTA,Proteinase K, etc.) strongly inhibit Taq DNA Polymerase, ethanol precipitation of DNA and repetitive treatments of DNA pellets with 70% ethanol is usually effective in removing traces of contaminants from the DNA sample.

Primers

Guidelines for primer selection:

PCR primers are usually 15-30 nucleotides in length. Longer primers provide sufficient specificity.

The GC content should be 40-60%. The C and G nucleotides should be distri -buted uniformly within the full length of the primer. More than three G or C nucleotides at the 3'-end of the primer should be avoided, as non specific priming may occur. The primer should not be self-complementary or comple -mentary to any other primer in the reaction mixture, in order to avoid primer -dimer and hairpin formation.

The melting temperature of flanking primers should not differ by more than 5°C, so the GC content and length must be chosen accordingly.

All possible sites of complementarity between primers and the template DNA should be noted.

If primers are degenerate, at least 3 conservative nucleotides must be located at the primer's 3'-end.

Estimation of the melting and annealing temperatures of primer:

If the primer is shorter than 25 nucleotides, the approx. melting temperature (Tm) is calculated using the following formula: Tm= 4 (G + C) + 2 (A + T)

G, C, A, T - number of respective nucleotides in the primer.

Annealing temperature should be approx. 5°C lower than the melting tem- perature.

If the primer is longer than 25 nucleotides, the melting temperature should be calculated using specialized computer programs where the interactions of adjacent bases, the influence of salt concentration, etc. are evaluated.

Primer concentration

The recommended concentration range is 0.1-1.0uM. Lower primer con- centrations may result in lower PCR yield, while higher primer concentrations increase the risk of non-specific amplification.

Initial DNA Denaturation / Enzyme Activation

The complete denaturation of the DNA template at the start of the PCR reaction is of key importance. Incomplete denaturation of DNA results in the inefficient utilization of template in the first amplification cycle and in a poor yield of PCR product. The initial denaturation should be performed over an interval of 1-3min at 95°C if the GC content is 50% or less. This interval should be extended up to 10min for GC-rich templates. If the initial denaturation is no longer than 3min at 95°C, Taq DNA Polymerase can be added into the initial reaction mixture. If longer initial denaturation or a higher temperature is necessary, Taq DNA Polymerase should be added only after the initial denaturation, as the stability of the enzyme dramatically decreases at temperatures over 95°C.

DNA Denaturation

In most conditions, a 0.5-1min DNA denaturation at 95°C is sufficient. For GC- rich DNA templates, this step may be increased to 3-4min. DNA denaturation can also be enhanced by the addition of either 10-15% glycerol or 10% DMSO, 5% formamide or 1.7-2M Betaine. The melting temperature of the primer-template complex decreases significantly in the presence of these reagents. Therefore, the annealing temperature must be adjusted accordingly. Additionally, 10% DMSO and 5% formamide inhibit DNA polymerase activity by 50%. Thus, the amount of enzyme should be increased, if these additives are used.

Primer Annealing

In most conditions, the annealing temperature should be 5°C lower than the primer -template melting temperature(Tm). Annealing for 0.5-1min is usually sufficient. If non-specific PCR products appear, the annealing temperature should be optimized stepwise in 1-2°C increments. The annealing temperature must also be adjusted when additives (glycerol, DMSO, formamide or Betaine), which change the melting temperature of the primer-template complex are used.

Extension

The extension step is performed at 72°C. As a general rule, the extension time is 1min per 1kb of the DNA fragment.

Number of Cycles

The number of cycles may vary depending on the amount of template DNA in the PCR mixture and the expected yield of PCR product. If less than 10 copies of the tem -plate are present in the reaction, approximately 40 cycles are required. With higher template amounts 25-35 cycles are sufficient.

Final Extension

After the last cycle, it is recommended to incubate the PCR mixture at 72°C for 5-15 min to fill-in the protruding ends of reaction products. If the PCR product is to be cloned into TA vectors, the final extension step can be extended to 30min.

GenDEPOT PO Box 454 Barker, Tx 77413 tech@gendepot.com