Taq Plus DNA Polymerase

Catalog No: P2024 (1000U)

Concentration: 2,5U/µl

Contents:

Taq Plus DNA Polymerase	400µl
10xPCR Buffer(Mg^{2+} plus)	1.25mlx4
dNTPs (2.5mM each)	1ml×4
6xLoading Buffer	1ml

Store at -20°C

In total 4 vials.

Description

Taq Plus DNA Polymerase is a mixture of Taq and Pfu polymerase, blends the processivity of Taq with the high fidelity of Pfu. The two enzymes act synergistically during PCR to generate more accurate and longer PCR products with greater yields compared to Taq DNA Polymerase alone. It can amplify DNA target up to 20 kb (simple template). And it is suitable as a direct replacement for ordinary Taq polymerase in most applications. PCR products used by Taq plus generate a mixture of blunt ends and single base (A)-3′ overhang. The error rate of this PCR amplification is $7.5x10^{-5}$ per nucleotide per cycle.

Unit Definition

One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nmole of dNTPs into an acid-insoluble form in 30 minutes at 70°C using hering sperm DNA as substrate.

Storage Buffer

20mM TrisCl (pH 8.0), 100mM KCl, 3mM $MgCl_2$ 1mM DTT, 0.1% NP-40 ,0.1% Tween20, 0.2mg/ml BSA, 50% (v/v) glycerol

10X PCR Buffer with Mg^{2+}

100 mM Tris-HCl pH 8.8, 500mM KCl, 1% Triton-X-100,

16mM $MgCl_2$

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Applications

- Amplification of long template up to 20kb
- Amplification of complex template
- High fidelity PCR

Basic PCR Protocol

The following basic protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (incubation time and temperature, concentration of Taq Plus DNA Polymerase, primers, $MgSO_4$, and template DNA) vary and need to be optimized.

1. Add the following components to a sterile microcentrifuge tube sitting on ice:

1.1 Recommended PCR assay with PCR Buffer (Mg^{2+} plus)

Reagent	Quantity, for 50µl reaction	Final concentration
Sterile deionized water	variable	-
10X PCR Buffer (Mg^{2+} plus)	5µl	1X
dNTPs （10mM each）	1µl	0.2 mM each
Primer I	variable	0.4-1µM
Primer II	variable	0.4-1µM
Taq Plus DNA Polymerase（2.5U/µl）	0.5-1 µl	1.25-2.5U/50 µl

Template DNA	variable	10pg-1µg
Total		50µl

1.2 Recommended PCR assay with PCR Buffer (Mg^{2+} free)

Reagent	Quantity, for 50µl reaction	Final concentration
Sterile deionized water	variable	-
10X PCR Buffer (Mg^{2+} free)	5µl	1X
dNTPs （10mM each）	1µl	0.2 mM each
Primer I	variable	0.4-1µM
Primer II	variable	0.4-1µM
25mM Mg^{2+}	variable	1-4mM
Taq Plus DNA Polymerase（2.5U/µl）	0.5-1 µl	1.25-2.5U/50 µl
Template DNA	variable	10pg-1µg
Total		50µl

Table for selection of 25 mM $MgCl_2$ solution volume in 50µl reaction mix:

Final Mg^{2+} conc.	1.0mM	1.5mM	2.0mM	2.5mM	3mM	4mM
Mg^{2+} Stock	2µl	3µl	4µl	5µl	6µl	8µl

Recommendations with Template DNA in a 50µl reaction volume

Human genomic DNA	0.1 µg-1 µg
Plasmid DNA	0.5 ng-5 ng

Phage DNA	0.1 ng-10 ng
E.coli genomic DNA	10 ng-100 ng

2. Mix contents of tube. Cap tubes and centrifuge briefly to collect the contents to the bottom.

When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µl mineral oil.

3. Perform 25-35 cycles of PCR amplification as follows:

Initial Denaturation	94°C	3 minutes
25-35 Cycles	94°C 55-68°C 72°C	30 seconds 30 seconds 2-5 minutes
Final Extension	72°C	10 minutes

4. Incubate for an additional 10 min at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.

5. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide staining. Use appropriate molecular weight standards.

Notes on cycling conditions

- The half-life of the enzyme is >40 minutes at 95°C.
- The error rate of Taq Plus DNA Polymerase in PCR is about $1x10^{-5}$ errors per nt per cycle; the accuracy (an inverse of error rate) an average number of correct nucleotides incorporated before making an error is $3.8x10^{-5}$ (determined according to the modified method described in).
- Taq Plus DNA Polymerase accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.
- The PCR products are the mixture of 3'-dA overhangs and blunt-ended products. But blunt-ended is the main product.
- The number of PCR cycles depends on the amount of template DNA in the reaction mix and on the expected yield of the PCR product. 25-35 cycles are usually sufficient for the majority PCR reaction. Low amounts of starting template may require 40 cycles.

Guidelines for preventing contamination of PCR reaction

During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set up.
- Use reagent containers dedicated for PCR. Use positive displacement pipettes, or use pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.
- Always perform "no template control" (NTC) reactions to check for contamination.

Quality Control

The absence of endodeoxyribonucleases, exodeoxyribonucleases and ribonucleases is confirmed by appropriate quality tests. Functionally tested in amplification of a single-copy gene from human genomic DNA.

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to a nicked DNA was observed after incubation of 10U Taq Plus DNA Polymerase with 1µg pBR322 DNA for 4 hours at 37°C and 70°C.

Exodeoxyribonuclease Assay

No detectable degradation of lambda DNA-HindIII fragments was observed after incubation of 10U Taq Plus DNA Polymerase with 1µg digested DNA for 4 hours at 37°C and 70°C.

Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 10U Taq Plus DNA Polymerase with 1µg E.coli [3H]-RNA (40000cpm/µg) for 4 hours at 37°C and 70°C.