Taq Plus DNA Polymerase

#P1031, 250U

Concentration: 2.5U/μl
Contents:

Taq Plus DNA Polymerase	100 μl
10× PCR Buffer (Mg^{2+} Plus)	1.25 ml
6× Loading Buffer	1 ml

Store at -20°C

For research use only.

In total 3 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. The elongation rate is 3kb/min. 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 amplified by Taq Plus are mixture of blunt-ends and 3'dA-overhangs. The error rate of this PCR amplification is 1.0×10^{-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 Tris-HCl (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.

10× PCR Buffer with Mg^{2+}

100mM Tris-HCl (pH 8.8), 500mM KCl, 1% Triton X-100, 16mM $MgSO_4$.

Applications

- Amplification of long template up to 20 kb

- 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, Mg^{2+}, 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	Final concentration
Sterile deionized water	variable	-
10× PCR Buffer (Mg^{2+} plus)	5 µl	1×
dNTPs (10mM each)	1 µl	0.2mM each
Primer I	variable	0.4-1µM
Primer II	variable	0.4-1µM
Taq Plus DNA Polymerase (2.5U/µl)	0.5-1.0 µl	1.25-2.5U/50 µl
Template DNA	variable	10pg-1µg
Total		50 µl

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

Reagent	Quantity	Final concentration
Sterile deionized water	variable	-
10× PCR Buffer (Mg^{2+} free)	5 µl	1×
dNTPs (10mM each)	1 µl	0.2mM each
Primer I	variable	0.4-1µM
Primer II	variable	0.4-1µM
25mM Mg^{2+}	variable	1.0-4.0mM
Taq Plus DNA Polymerase (2.5U/µl)	0.5-1.0 µl	1.25-2.5U/50 µl
Template DNA	variable	10pg-1µg
Total		50 µl

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

Final Mg^{2+} Conc. (mM)	1.0	1.5	2.0	2.5	3.0	4.0
Mg^{2+} (25mM)	2 µl	3 µl	4 µl	5 µl	6 µl	8 µl

Recommendation amounts of template DNA in a 50 µl reaction mix:

Human genomic DNA	0.1µg-1µg
Plasmid DNA	0.5ng-5ng
Phage DNA	0.1ng-10ng
E.coli genomic DNA	10ng-100ng

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	95°C	3 minutes
25-35 Cycles	95°C 55-68°C 72°C	30 seconds 30 seconds 1-5 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 nucleic acid dye staining. Use appropriate molecular weight standards.

Notes on cycling conditions

- The half-life of enzyme is >40 minutes at 95°C.
- The error rate of Taq Plus DNA Polymerase in PCR is 1.0×10^{-5} errors per nt per cycle; the accuracy (an inverse of the error rate), an average number of correct nucleotides incorporated before making an error, is 3.8×10^{-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.

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.