Taq Plus DNA Polymerase

Ref:	**NB-03-0097**	**250 U**
	NB-03-0098	**250 U**
	NB-03-0099	**1 000 U**
	NB-03-0100	**1 000 U**

Contents

NB-03-0097	
Taq plus DNA Polymerase (2.5 U/µl)	100 µl
10X PCR Buffer (Mg^{2+} Plus)	1.4 ml
6X Loading Buffer	1 ml

NB-03-0098*	
Taq plus DNA Polymerase(2.5 U/µl)	100 µl
10X PCR Buffer (Mg^{2+} Plus)	1.4 ml
dNTPs (each 2.5 mM)	1 ml
6X Loading Buffer	1 ml

NB-03-0099	
Taq plus DNA Polymerase (2.5 U/µl)	400 µl
10X PCR Buffer (Mg^{2+} Plus)	1.4 ml ×2
6X Loading Buffer	1 ml

NB-03-0100*	
Taq plus DNA Polymerase (2.5 U/µl)	400 µl
10X PCR Buffer (Mg^{2+} Plus)	1.4 ml ×2
dNTPs (each 2.5 mM)	1 ml ×2
6X Loading Buffer	1 ml

*** with dNTPs**

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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.

Applications

Amplification of long template up to 20kb

High fidelity PCR

Definition of Activity Unit

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

Storage Buffer

20mM TrisCl (pH8.0), 100mM KCl, 3mM MgCl2 1mM DTT, 0.1% NP-40 ,0.1% Tween20, 0.2mg/ml BSA, 50% (v/v) glycerol

10X Taq Plus Buffer with $MgSO_4$

120mM Tris-HCl(PH 8.8), 500mMKCl, 1%Triton-X-100, 100mM Lycine, 25mM MgSO4

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, MgSO4, and template DNA) vary and need to be optimized.

1. Add the following components to a sterile microcentrifuge tube sitting on ice: recommended PCR assay with Taq Plus Buffer (Mg2+ plus)

Reagent	Quantity for 50µl of reaction mixture	Final Concentration
Sterile deionized water	variable	-
10X Taq Plus Buffer (Mg^{2+} plus)	5 µl	1X
dNTPs (10mM each）	1 µl	0.2 mM each
Primer I	variable	0.4 - 1 µM

Primer II	variable	0.4 - 1 µM
Taq Plus DNA Polymerase (5U/µl)	0.25 - 0.5 µl	1.25 - 2.5U/50 µl
Template DNA	variable	10pg-1µg
Total		50 µl

Recommandations with Template DNA in a 50 µl 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℃	3 minutes
25-35 cycles	94℃ 55-68℃ 72℃	30 seconds 30 seconds 1 minute
Final extension	72℃	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.

Note:

- The half-life of 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.

Store all components at –20°C