# 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**

## Note

10X PCR Buffer ($Mg^{2+}$ Plus) can replace with 10×PCR Buffer ($Mg^{2+}$ free) and 25 mM $MgCl_2$. Please choose the appropriate package for your experiment.

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## Description

Taq Plus, a mixture of taq and pfu polymerase, blends the processivity of taq with the high fidelity of pfu. Therefore, this specially formulated Taq plus allow amplification of the higer fidelity and longer templates than the single-enzyme formulations. It is also a better choice for amplifying complex template, such as GC-rich template. And it is suitable as a direct replacement for ordinary Taq Polymerase in most applications. In addition, Using Taq plus results in 3´-dA overhangs PCR products, which can be used in TA clone.

## Features

**High fidelity**: Its fidelity is four times higher than ordinary Taq polymerase.

**High yields**: Suitable for amplifying large fragment, suitable for complex template which is rich in GC or repeat sequence.

## Applications

- Long PCR with high fidelity
- Amplification reaction of complex template

## Quality Control

The absence of endodeoxyribonucleases, exodeoxyribonucleases and ribonucleases confirmed by appropriate quality tests

Functionally tested in 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

20 mM Tris-HCl (pH 8.0), 1 mM DTT, 0.1 mM EDTA, 100 mM KCl, 0.5 % TW 20, 0.5 % NP 40, 50 % Glycerol

## 10X Taq Plus Buffer with $MgSO_4$

200mM Tris-HCl (pH 8.8， 25℃), 100mM KCl, 100mM (NH4)2SO4, 20mM MgSO4, 1.0% Triton®X-100, 1mg/ml BSA

## 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, $MgCl_2$, 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 Taq Plus Buffer ($Mg^{2+}$ 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 |

1.2. Recommended PCR assay with Taq Plus Buffer ($Mg^{2+}$ free)

| Reagent | Quantity for 50µl of reaction mixture | Final Concentration |
|---|---|---|
| Sterile deionized water | variable | - |
| 10X Taq Plus Buffer ($Mg^{2+}$ free) | 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 |
| 25 mM $Mg^{2+}$ | variable | 1 - 4 mM |
| Taq Plus DNA Polymerase (5U/µl) | 0.25 - 0.5 µl | 1.25 - 2.5U/50 µl |
| Template DNA | variable | 10pg-1µg |

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

| Final $Mg^{2+}$ conc. | 1.0 mM | 1.5 mM | 2.0 mM | 2.5 mM | 3 mM | 4 mM |
|---|---|---|---|---|---|---|
| $Mg^{2+}$ Stock | 2 µl | 3 µl | 4 µl | 5 µl | 6 µl | 8 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°C | 3 minutes |
| 25-35 cycles | 94°C<br>55-68°C<br>72°C | 30 seconds<br>30 seconds<br>1 minute |
| 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. Notes on cycling conditions :

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**