

Plus Mix (2x)

Ref: NB-03-0101 1 ml
NB-03-0102 5x 1ml

Contents

NB-03-0101 (40 reactions of 50 µl each)	
2×PCR Plus Mix	1 ml
Nuclease-free Water	1 ml

NB-03-0102 (200 reactions of 50 µl each)	
2×PCR Plus Mix	5x 1 ml
Nuclease-free Water	5x 1 ml

Description

Plus 2X Mix is a premixed, ready-to-use solution containing Plus DNA Polymerase, dNTPs, MgCl₂ and Reaction Buffer at optimal concentrations for efficient amplification of DNA templates by PCR. To prepare the final PCR, only primers and template DNA are added. Plus Mix contributes to highly reproducible PCR by reducing the risk of pipetting errors, miscalculation and contamination. It also contributes to higher sensitivity by adding enhancer.

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 higher 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

Convenient : only primers and template DNA are added when prepare final PCR

High efficiency: saving your time by simplifying the process

Reproducible: reducing the risk of pipetting errors, miscalculation and contamination

Applications

- Long PCR with high fidelity
- High reproducible PCR for complex templates

- High throughput PCR for complex templates
- Generation of PCR products for TA cloning

Unit Definition

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.

2×PCR Plus Mix

20 mM Tris-HCl (pH 8.0), 100 mM KCl, 3 mM MgCl₂, 400 mM dNTPs, 0.1 U/μl Plus DNA Polymerase, bromophenol blue, other intensifier and stabilizing agent
Store at -20°C.

Basic PCR Protocol

All solutions should be thawed on ice, gently vortexed and briefly centrifuged.

1. Add in a thin walled PCR tube on ice:

Components	Volume	Final Concentration
2x PCR Plus Mix	25 μl	1 x
Primer mix (10 μM each)	4 μl	0.4μM each
Template DNA	1-10 μl	n/a
Nuclease-Free Water	To 50 μl	n/a

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 and overlay with 50 μl of mineral or silicone oil.
3. Cap tubes and centrifuge briefly to collect the contents to the bottom.

4. Perform PCR using the following thermal cycling conditions:

Initial Denaturation	94°C	3 minutes
25-35 cycles	94°C	45 seconds
	55°C	30 seconds
	72°C	1.5 min
Final extension	72°C	10 minutes

4. 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 :

- Initial denaturation can be performed over an interval of 1-5 min at 95°C depending on the GC content of template.
- Optimal annealing temperature is 5°C lower than the melting temperature of primer-temperature DNA duplex. If nonspecific PCR products are obtained optimization of annealing temperature can be performed by increasing temperature stepwise by 1-2°C.
- 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.
- The time of the final extension step can be extended for amplicons that will be cloned into T/A vectors.