NucleoGene High-Fidelity PCR Master Mix (2X)

Instructions for Use

Release Date— 01.01.2022

Store at -20°C

REF

For research use only.
Not suitable for diagnostic use.
For professional use only.

NGMM027-20 μ l x 200 rxn
20 μ l x 500 rxn
20 μ l x 1000 rxn

Description

NucleoGene HiFi DNA Polymerase is a new generation of ultra-fidelity DNA polymerase based on Pfu DNA Polymerase. It has high amplification efficiency and wide template adaptability, and is suitable for almost all PCR reactions. NucleoGene HiFi DNA Polymerase is capable of amplifying long fragments such as 40 kb λ DNA, 40 kb plasmid DNA, 20 kb genomic DNA and 10 kb cDNA. The amplification error rate of NucleoGene HiFi DNA Polymerase is 100-fold lower than that of conventional Taq and 10-fold lower than that of Pfu. In addition, NucleoGene HiFi DNA Polymerase has a good resistance to PCR inhibitors and can be used for direct PCR amplifications of bacteria, fungi, plant tissues, animal tissues, and even whole blood samples. NucleoGene HiFi DNA Polymerase has 5'→3' polymerase activity and 3'→5' exonuclease activity, and the amplified product is blunt-ended, suitable for fragment amplification of the seamless cloning kit and amplification of the second-generation sequencing library. NucleoGene High-Fidelity PCR Master Mix (2X) contains NucleoGene HiFi DNA Polymerase, dNTP, and an optimized buffer system. The amplification can start only with the addition of primer and template, thereby easing PCR setup and improving reproducibility.

Composition

Taq Pfu DNA polymerase, reaction buffer, MgCl2, dNTP (dATP, dCTP, dGTPand dTTP), stabilizers and enhancers.

Applications

• High fidelity PCR
•Long range PCR
•Site-directed mutagenesis
•Blunt end PCR cloning

PROTOCOL

1. Gently vortex and briefly centrifuge NucleoGene High-Fidelity PCR Master Mix (2X) after thawing.

2. Place a thin-walled PCR tube on ice and add the following components for each examplesreactions:

For a 20 μl reaction volume:

Component	Volume	Final Conc.
High-Fidelity PCR Master Mix (2X)	10 μl	1X
Forward primer (10μM)	1 μl	0.5 μM
Forward primer (10μM)	1 μl	0.5 μM
DNA template	variable	*
Nuclease-Free Water	to 20μl	N.A.

*Human genomic DNA : 10 ~ 100ng
Bacterial genomic DNA : 5 ~ 50ng
Purified plasmid or phage DNA : 1 ~ 5ng

3. Gently vortex the samples and spin down.

4. When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 20 μL of mineral oil.

General Guidelines for Amplification by PCR

NucleoGene High-Fidelity PCR Master Mix (2X) was functionally tested for PCR amplifications using the various primer sets (0.5 kb ~ 17kb) from human beta-globin gene.

A. Denaturation

Generally, a 2-minute initial denaturation step at 95°C is sufficient. Subsequent denaturation steps will be between 30 seconds and 1 minute.

B. Annealing

Optimize the annealing conditions by performing the reaction startingapproximately 5°C below the calculated melting temperature of the primers andincreasing the temperature in increments of 1°C to the annealing temperature.

The annealing step is typically 30 seconds to 1 minute.

C. Extension

The extension reaction is typically performed at the optimal temperature for Taq DNA polymerase, which is 72–74°C. Allow approximately 1 minute for every 1kb of DNA to be amplified. A final extension of 5 minutes at 72–74°C is recommended.

D. Refrigeration

Routine storage: -18 °C to -28 °C. Shipping and temporary storage for up to 1 month at room temperature or storage for up to 6 months at 2–8°C has no detrimental effects on the quality of the product.

E. Cycle Number

Generally, 25–40 cycles result in optimal amplification of desired products. Occasionally, up to 40 cycles may be performed, especially for detection oflow-copy targets.

Step	Temperature °C	Time	Number of cycles
Initial denaturation	95℃	3 min	1
Denaturation	98℃	20 sec	10-35
Annealing	Annealing Temp* 50-68℃	40 sec	
Extension	72℃	15-60 sec	
Final Extension	72℃	1 min	1

***Annealing Temp. = Tm – (4 ~ 6° C) Tm (Melting Temp.) = [4° C x (number of G + C)] + [2° C x (number of A + T)]**
-Extend the Initial denaturation time to 5 min for GC≧70% templates.

-High salt concentrations affect DNA solubilization; in order to ensure that the complex GC template can be completely denatured, adjust the denaturation temperature to 98 ° C. High salt buffer will also affect primer annealing, to determine the most suitable annealing temperature, incrementally increasing the annealing temperature by 3° C above the initial recommended 56° C.

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-For the two-step amplification programs, the recommended annealing/extension temperature range is 65-68 ° C, and the extension time should range between 30-60 sec/kb.

-Calculate the extension time as 15 sec/kb for target fragments ≦1kb under conventional conditions, and 30 sec/kb when the target fragment is >1kb or a higher final concentration of product is desired.

-To obtain higher fidelity, ensure the number of amplification cycles ≦ 25 cycles; increasing the number of amplification cycles, increasing polymerase error and mismatch rate.

If there are non-specific PCR amplifications, use an annealing temperature of 2~5 ° C higher than the calculated melting temperature.

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