NucleoGene Hot Start BLEND Green PCR Master Mix (2X)

Instructions for Use

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Store at -20°C

REF

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

NGMM028- 20µl x 250 rxn
20µl x 500 rxn
20µl x 1000 rxn

Description

Hot Start Blend Green PCR Master Mix is a 2X concentrated solution mixture of DNA Polymerase Green Dye, Buffer and dNTP Mixture. When in use, simply add the template and primers into this product to do PCR reaction, greatly simplifies the process of operation and operation reduces the PCR process contamination. Compared to average 2X Blend Green PCR Master Mix, this product have much faster higher sensitivity and higher amplification efficiency advantages. Hot Start Blend Green PCR Master Mix allows you to easily load the product into the gel after the PCR process is finished. You do not need any Loading Dye.Taq DNA polymerase extension rate is about 1-4kb/min, 3'end of PCR product with "A"base and can directly clone into TA vector.

Composition

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

Applications

- High throughput PCR.
- Routine PCR with high reproducibility.
- Generation of PCR products for TA. cloning.
- RT-PCR.

PROTOCOL

1. Gently vortex and briefly centrifuge Hot Start Blend Green PCR Master Mix (2X) after thawing.

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

For a 20 µl reaction volume:

Component	Volume	Final Conc.
Hot Start Master Mix (2X)	10 µl	1X
Forward primer (10µM)	0.25–2.5µl	0.1–1.0µM
Forward primer (10µM)	0.25–2.5µl	0.1–1.0µM
DNA template	1–5µl	<250ng
Nuclease-Free Water	to 20µl	N.A.

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 25 µL of mineral oil.

General Guidelines for Amplification by PCR

The following guidelines apply to target sequences between 200 and 2,000bp and are optimal for typical thermal cyclers.

A. Denaturation

Generally, a 15 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 starting approximately 5°C below the calculated melting temperature of the primers and increasing 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 3kb of DNA to be amplified.

A final extension of 5 minutes at 72–74°C is recommended.

D. Refrigeration

If the thermal cycler has a refrigeration or "soak" cycle, the cycling reaction can be programmed to end by holding the tubes at 4°C for several hours.

This cycle can minimize any polymerase activity that might occur at higher temperatures, although this is not usually a problem.

E. Cycle Number

Generally, 25–30 cycles result in optimal amplification of desired products. Occasionally, up to 40 cycles may be performed, especially for detection of low-copy targets.

Step	Temperature °C	Time	Number of cycles
Initial denaturation	95	2 min 30 sec	1
Denaturation	95	20-30 sec	30-45
Annealing	Primers Optimum Working TM	40 sec	
Extension/	72	1 min/kb	
Final Extension	72	5-15 min	1

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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 setup.

• Use reagent containers dedicated for PCR. Use positive displacement pipettes, or pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.

• Always perform “no template control” (NTC) reactions

Guidelines for Primer Design

• PCR primers are generally 15-30 nucleotides long.

• Differences in melting temperatures (Tm) between the two primers should not exceed 5°C.

• Optimal GC content of the primer is 40-60%. Ideally, C and G nucleotides should be distributed uniformly along the primer.

• Avoid placing more than three G or C nucleotides at the 3’-end to lower the risk of non-specific priming.

• If possible, the primer should terminate with a G or C at the 3’-end.

• Avoid self-complementary primer regions, complementarities between the primers and direct primer repeats to prevent hairpin formation and primer dimerization.

• Check for possible sites of undesired complementary between primers and template DNA.

• When designing degenerate primers, place at least 3 conservated nucleotides at the 3’-end.

Estimation of Primer Melting Temperature

For primers containing less than 25 nucleotides, the approx. melting temperature (Tm) can be calculated using the following equation:

Tm= 4 (G + C) + 2 (A + T),

Where G, C, A, T represent the number of respective nucleotides in the primer. If the primer contains more than 25 nucleotides we recommend using specialized computer programs, to account for interactions of adjacent bases, effect of salt concentration, etc.

Components of the Reaction Mixture Template DNA

Optimal amounts of template DNA for a 50 µL reaction volume are 0.01-1 ng for both plasmid and phage DNA, and 0.1-1 µg for genomic DNA. Hi her amounts of template increase the risk of generation of non-specific PCR products. Lower amounts of template reduce the accuracy of the amplification.

Trace amounts of certain agents used for DNA purification, such as phenol, EDTA and proteinase K, may inhibit DNA polymerase. Ethanol precipitation and repeated washes of the DNA pellet with 70% ethanol usually removes trace contaminants from DNA samples.

Primers

The recommended concentration range of the PCR primers is 0.1-1 µM. Excessive primer concentrations increase the probability of mispriming and generation of non-specific PCR products. For degenerate primers and primers used for long PCR we recommend higher primer concentrations in the range of 0.3-1 µM.

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