Gloria Nova HS 2X HF Master Mix

Catalog: RK20715
Size: 50 RXN / 200 RXN (50 μL/RXN)
Concentration: 2X

Gloria Nova HS 2X HF Master Mix	RM20384

Product Description

Gloria Nova HS DNA polymerase is an ideal enzyme for high fidelity PCR with excellent processivity. It is a novel engineered enzyme with comparable performance to *Pyrococcus furiosus* DNA polymerase. With unique structure Gloria Nova HS DNA polymerase contains a recombinant synthesis enhancement domain to increase fidelity and extension speed. The antibody-mediated hot-start feature significantly inhibits non-specific amplifications at room temperature.
Gloria Nova is one of the thermostable DNA polymerases with strong 3'-5' exonuclease activity (proofreading activity), which results in its extreme high fidelity, 10-15 times higher than *Taq* DNA polymerase and 6 times higher than *Pyrococcus furiosus* DNA polymerase.
The Gloria Nova HS 2X HF Master Mix contains Gloria HiFi DNA polymerase, dNTPs, reaction buffer, and optimized $MgCl_2$. It is an ideal product with good amplification efficiency for diversity templates including animals, plants, cDNA, etc.

Storage

Upon receipt, store all components at -20°C.

Thermal Inactivation

None

Product End

Blunt end

Standard Protocol

- It is recommended to prepare all reaction components on ice, and then quickly transfer the reaction system to a thermocycler preheated to 98 ° C.
- All components should be mixed and collected at the bottom of a tube with a quick spin before use. Add Gloria Nova HS 2X HF Master Mix at the end to prevent primer degradation by its strong 3´-5´ exonuclease activity.
- Note: The Gloria Nova HS 2X HF Master Mix require special reaction conditions different from other polymerase protocols. Please refer to the recommended reaction conditions below for the better amplification yields.

Recommended Reaction

Components	25 μL	50 μL	Total Concentration
Gloria Nova HS 2X HF Master Mix	12.5μL	25 μL	1X
Forward Primer (10 μM)	0.5 μL	1 μL	0.2 μM
Reverse Primer (10 μM)	0.5 μL	1 μL	0.2 μM
DNA Template*	Variable	Variable	<300 ng
Nuclease-free Water	to 25 μL	to 50 μL	N/A

** Note : The optimal reaction concentration varies with different DNA templates. Please refer to the basic principles of PCR below.*

Recommended PCR Program

Step	Temp	Time	Cycles
Pre-denaturation	98°C	45 s	1
Denaturation	98°C	10 s	25-35
Annealing	55-65°C	20-30 s	
Extension	72°C	10-30 s/kb	
Post-extension	72°C	1-5 min	1
Hold	4-12°C	∞	1

PCR Principles

1. Template

High-quality purified DNA templates are important to high-fidelity PCR reactions. The recommended DNA template amounts with different complexity are listed below.

Recommended Input (For a 50μL reaction)

DNA	Input Amount
Plants, animals and human gDNA	10 ng-300 ng
E.coli, lambda genome	10 ng-100ng
Plasmid DNA	1 pg-10 ng

Note: If the DNA template is obtained from a cDNA synthesis reaction, the template volume should be less than 10% of the total reaction volume. If long fragments are amplified, the amount of template input should be increased appropriately.

2. Primers

Oligonucleotide primers are typically 20-40 nucleotides in length with a GC content of 40-60%. Primers can be designed and analyzed using software such as Primer 3. The final concentration of each primer in the PCR reaction system should be in the range of 0.2-1 μM.

3. Denaturation

98 ° C pre-denaturation for 45 s can fully denature most DNA templates. In the case of high complexity DNA templates, the pre-denaturation time should be extended up to 3 minutes for fully denaturation. Generally, the recommended denaturation condition for low-complexity DNA templates is 98 ° C, 5-10 s.

4. Annealing

The annealing temperature of Gloria Nova HS DNA polymerase is usually higher than other PCR polymerases. Generally, primers longer than 20 nt are annealed at (lower primer Tm+3) ° C for 10-30 s; when the primers are shorter than 20 nt, an annealing temperature equivalent to the lower primer Tm should be used. When using a new primer set for PCR reaction, we recommend a gradient PCR to determine the optimal annealing temperature. In a two-step amplification protocol, the annealing temperature should be set to the extension temperature.

5. Extension

The recommended extension temperature is 72 ° C.

The extension time depends on the length and complexity of the amplicon. For the low-complexity amplicons (plasmid DNA), the extension condition is 10-30 s / kb. For high-complexity amplicons, such as genomic DNA, it is recommended to increase the extension time to 1 min / kb. In some cases, the extension time for cDNA templates should be less than 1 min / kb.

6. Cycles

To obtain enough yield of PCR products, 25-35 cycles are recommended.

7. PCR Products

Gloria Nova HS DNA polymerase produces blunt-end PCR products, which might be directly used in the sequential blunt-end cloning. For T/A cloning, the PCR products should be further purified to remove Gloria Nova HS DNA polymerase before dA tailing reaction, because the proofread activity of Gloria Nova HS DNA polymerase will remove the dA-overhangs. Taq DNA polymerase (ABclonal RK20600) or Klenow exo-(ABclonal RK20526) are recommended to dA tailing reaction for the purified DNA products.