Gloria Nova HS 2X Master Mix with Dye

Cat.No. : RK20728

Product Components

Components	Component Number	Size-1 1 mL	Size-2 5 mL
Gloria Nova HS 2X Master Mix with Dye	RM20394	1 mL	1 mL × 5

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 a unique structure. Gloria Nova HS DNA polymerase contains a recombinant synthetic enhanced 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 extremely high fidelity approximately 100x that of conventional Taq. The Gloria Nova HS 2X Master Mix with Dye is an ideal product with good amplification efficiency for diversity templates including animals, plants, cDNA, etc.

Gloria Nova HS 2X Master Mix with Dye contains Gloria Nova HS DNA polymerase, dNTP, and an optimized buffer system. It only needs to add primers and templates to perform amplification, thereby reducing pipetting operations, significantly controlling cross-contamination among samples, and improving detection throughput and reproducibility of results.The amplification system contains protective agents that keep Gloria Nova HS 2X Master Mix with Dye stable in activity after repeated freezing and thawing. Gloria Nova HS 2X Master Mix with Dye contains loading buffer, PCR products can be directly loaded for electrophoresis after the reaction.

Storage

-20℃

5′-3′exonuclease activity

No

3′-5′exonuclease activity

Yes

Product End

Blunt end

Operation Description

Standard Protocol

1. It is recommended to prepare all reaction components on ice, and then quickly transfer the reaction system to a thermocycler preheated to 98℃.
2. All components should be mixed and collected at the bottom of a tube with a quick spin before use. Add Gloria Nova HS 2X Master Mix with Dye at the end to prevent primer degradation by its strong 3′-5′ exonuclease activity.

Note: The Gloria Nova HS DNA polymerase requires special reaction conditions different from other polymerase protocols. Please refer to the recommended reaction conditions below for the better amplification yields.

Recommended Reaction:

Component	25 μL Reaction	50 μL Reaction	Final Concentration
Gloria Nova HS 2X Master Mix with Dye*	12.5 μL	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: Gloria Nova HS 2X Master Mix with Dye contains loading buffer, the PCR products can be used directly for agarose gel electrophoresis, without addition of DNA Loading Buffer.

** 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
Initial Denaturation	98℃	3 min	1
Denaturation	98℃	10 s	25-35
Annealing	55-65℃	20-30 s	
Extension	72℃	30-60 s/kb*	
Final Extension	72℃	1-5 min	1
Hold	4-12℃	-	1

*Note: Properly extending the extension time can improve the amplification yield. For complex amplification templates, such as genomic DNA, it is recommended to extend at a speed of 60 s/kb, and more recommended conditions please refer to the basic principles of PCR below.

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 (For a 50 μL reaction):

DNA	Input Amount
Plants, animals and human gDNA	10 ng-100 ng
E.coli, lambda gDNA	500 pg-200 ng
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 0.1-1 μM.

3. Denaturation

The DNA template can fully denaturation when the initial denaturation time is set to 3 min. Generally, the recommended denaturation condition for low-complexity DNA templates is 98℃, 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)℃ for 10-30 s; when the primers are shorter than 20 nt, an annealing temperature equivalent to the lower primer Tm. 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. Extention

The recommended extension temperature is 72℃. The extension time depends on the length and complexity of the amplicon. For the low-complexity amplicons (plasmid DNA), the extension condition is 30 s/kb. For high-complexity amplicons, it is recommended to increase the extension time to 60 s/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.

8. Complex templates

For complex templates that cannot be amplified by conventional PCR (such as long fragments, uneven Tm distribution, templates with special structures), you can try the two-step method or the touchdown method.

Recommended Two-Steps PCR Program

Step	Temp	Time	Cycles
Initial Denaturation	98℃	3 min	1
Denaturation	98℃	10 s	25-35
Annealing/Extension*	65-72℃	60 s/kb	
Final Extension	65-72℃	1-5 min	1
Hold	4-12℃	-	1

*Note: In general, 68℃ is recommended, but it can be changed according to the Tm value.

Recommended Touchdown PCR Program

Step	Temp	Time	Cycles
Initial Denaturation	98℃	3 min	1
Denaturation	98℃	10 s	5
Annealing	74℃	60 s/kb	
Denaturation	98℃	10 s	5
Annealing	72℃	60 s/kb	
Denaturation	98℃	10 s	5
Annealing	70℃	60 s/kb	
Denaturation	98℃	10 s	25
Annealing	68℃	60 s/kb	
Final Extension	72℃	1-5 min	1
Hold	4-12℃	-	1