Gloria Nova HS PCR Kit

Cat.No. : RK20714

ABclonal Technology

Product Components

Components	Component Number	Size-1 50 RXN(50 μL/RXN)	Size-2 200 RXN (50 μL/RXN)
Gloria Nova HS DNA Polymerase (2,000 U/mL)	RM20405	50 μL	200 μL
5X Gloria Nova HF Buffer	RM20182	500 μL	1 mL × 2
2.5X Gloria Nova GC Buffer	RM20183	1 mL	1 mL × 4
dNTPs (10 mM each)	RM20120	50 μL	200 μL

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 PCR Kit is supplied with a 5X Gloria Nova HF Buffer and a 2.5X Gloria Nova GC Buffer. The 5X Gloria Nova HF Buffer is an optimized buffer for general high fidelity amplifications while the 2.5X Gloria Nova GC Buffer is used in the amplifications of problematic or GC-rich templates.

Storage

Upon receipt, store all components at -20℃.

Thermal Inactivation

No

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℃.It is recommended to prepare all reaction.

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

5X Gloria Nova HF Buffer Reaction System

Component	25 μL Reaction	50 μL Reaction	Final Concentration
5X Gloria Nova HF Buffer	5 μL	10 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
dNTPs (10 mM)	0.5 μL	1 μL	0.2 mM
Gloria Nova HS DNA Polymerase	0.5 μL	1 μL	2 U/50 μL
ddH_2O	to 25 μL	to 50 μL	N/A

2.5X Gloria Nova GC Buffer Reaction System

Component	25 μL Reaction	50 μL Reaction	Final Concentration
2.5X Gloria Nova GC Buffer	10 μL	20 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
dNTPs (10 mM)	0.5 μL	1 μL	0.2 mM
Gloria Nova HS DNA Polymerase	0.5 μL	1 μL	2 U/50 μL
ddH_2O	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
Initial Denaturation	98℃	45 s	1
Denaturation	98℃	10 s	25-35
Annealing	55-65℃	20-30 s	
Extension	72℃	10-30 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-300 ng
E.coli, lambda gDNA	10 ng-100 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. Enhancer

The Enhancer solution is an optional component to increase the amplification efficiency for problematic templates, such as GC-rich sequence or genes with strong secondary structure. Note:Since the enhancer is included in the 2.5X Gloria Nova GC Buffer, additional enhancer is not recommended with the use of 2.5X Gloria Nova GC Buffer. Excess amount of enhancer may be inhibitory.

4. Buffer

The Gloria Nova HS PCR Kit contains a 5X Gloria Nova HF Buffer and a 2.5X Gloria Nova GC Buffer.The 5X Gloria Nova HF Buffer is designed forgeneral high fidelity PCR amplification , and the 2.5X Gloria Nova GC Buffer is optimized for the amplifications of GC-rich templates.

5. Denaturation

98℃ 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℃, 5-10 s.

6. 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 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.

7. 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 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.

8. Cycles

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

9. PCR Products

Gloria Nova HS DNA polymerase produces blunt-end PCR products, which might be directly used in the sequential blunt-end cloning.