

## Product Components

Components	Component Number	Size-1	Size-2
		1 mL	5 mL
Gloria Nova HS 2X Master Mix V2	RM20399	1 mL	1 mL × 5

## Product Description

Gloria Nova HS DNA polymerase is an ideal enzyme for high fidelity PCR. It is a novel engineered enzyme with a unique structure, incorporating a recombinant synthetic enhanced domain to increase fidelity and extension speed. The antibody-mediated hot-start mechanism effectively inhibits non-specific amplifications at room temperature. Gloria Nova offers excellent fidelity and is one of the highest-fidelity thermostable DNA polymerases available. The Gloria Nova HS 2X Master Mix V2 is an ideal product with good amplification efficiency for diversity templates including animals, plants, cDNA, etc.

Gloria Nova HS 2X Master Mix V2 contains Gloria Nova HS DNA polymerase, dNTP, and an optimized buffer system. Only primers and templates needed to be added for amplification, minimizing pipetting steps, significantly controlling cross-contamination among samples, and improving detection throughput and reproducibility of results. The formulation includes protective agents that ensure stable enzymatic activity even after repeated freeze-thaw cycles.

## Storage

-20°C

## 5′-3′ exonuclease activity

No

## 3′-5′ exonuclease activity

Yes

## Product End

Blunt end

## Operation Description

### Standard Protocol

- It is recommended to prepare all reaction components on ice, and then quickly transfer the reaction system to a thermocycler preheated to 95°C.
- 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 V2	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: The optimal reaction concentration varies with different DNA templates. The recommended DNA template amounts with different complexity are listed below (For a 50  $\mu$ L reaction):

DNA	Input Amount
Plants, animals and human gDNA	10 ng-100 ng
<i>E.coli</i> , lambda gDNA	500 pg-200 ng
Plasmid DNA	1 pg-10 ng
cDNA	1 - 5 $\mu$ L(should be less than 10% of the total reaction volume)

#### Recommended PCR Program:

Step	Temp	Time	Cycles
Initial Denaturation	95°C	3 min	1
Denaturation	95°C	15 s	} 25-35
Annealing	60°C*	30 s	
Extension	72°C	10 s/kb**	
Final Extension	72°C	5 min	1
Hold	4-12°C	-	1

\*Note: In general, the annealing temperature can be set to 60°C by default, and for primers longer than 20 nt, annealing temperature should be set to (lower primer  $T_m+3$ )°C, and for primers shorter than 20 nt, the annealing temperature should be equivalent to the lower primer  $T_m$ .

\*\*Note: Properly extending the extension time can improve the amplification yield. Generally, it can be extended at a speed of 10 s/kb. For more complex templates or fragments with a length of more than 10 kb, the extension time can be appropriately extended to 15 s/kb.

## FAQs

### 1. Low or no amplification yields

- Problems with PCR primers: setting up a positive control reaction.
- The annealing temperature is not suitable: adjust the annealing temperature and carry out gradient optimization experiments.
- Low primer concentration or insufficient cycle number in PCR system: increase the amount of primer appropriately, or increase the number of amplification cycles.
- Insufficient extension time: extend the extension time to 15 s/kb.
- Effect of inhibitors: use a high-purity template, or amplify after template dilution.

### 2. Generate non-specific amplified bands

- The primer concentration or DNA template concentration in the PCR system is too high: reduce the amount of primers and template appropriately.
- The annealing temperature is too low or the number of cycles is too high: gradient optimization experiments can be carried out by increasing the annealing temperature or decreasing the number of cycles.
- PCR primer mismatch: redesign PCR primers.