Gloria U HS 2X Master Mix

RK20722

Product components

Components	Cat.No	Size-1 1 mL	Size-2 5 × 1 mL
Gloria U HS 2X Master Mix	RM20393	1 mL	5 × 1 mL

Product Description

Gloria U hot-start DNA polymerase is a modified version of Gloria Nova DNA polymerase. It efficiently amplifies uracil-containing templates. Due to the inactivation of uracil-binding capacity of Gloria U HS DNA polymerase, it can efficiently amplify bisulfite-treated DNA, and the enzyme exhibits the same high yield as Gloria Nova hot-start DNA polymerase. Gloria U hot-start high-fidelity DNA polymerase has 5′-3′ polymerase and 3′→5′ exonuclease (corrected) activity, but no 5′-3′ exonuclease activity. The convenient 2X master mix format contains dNTPs, Mg++ and a proprietary buffer, and requires only the addition of primers and DNA template for robust amplification.

Storage

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

Notes

1. Use 25μL of Gloria U HS 2X Master Mix per 50 μL reaction volume. It is recommended to prepare the reaction system on ice and quickly transfer it to a PCR instrument pre-warmed at 98 °C.

2. The concentration of each dNTP in the reaction is 200 μM. dUTP can be added up to a maximum concentration of 200 μM.

3. All components should be mixed and centrifuged instantaneously before use. After mixing the other reaction components, Gloria U DNA Polymerase was added to prevent the degradation of primers by its 3 -5 exonuclease activity.

Standard Protocol

Recommended Reaction

Take 25 μL and 50 μL reaction systems as examples.

Components	25 μL	50 μL	Total Concentration
Gloria U 2X HS Master Mix	12.5 uL	25 μL	1X
Forward Primer (5 μM)	1 μL	2 μL	0.2 μM
Reverse Primer (5 μM)	1 μL	2 μL	0.2 μM
DNA Template*	Variable	Variable	< 300 ng
Nuclease-free Water	to 25 μL	to 50 μL	N/A

Note: Gently mix the reaction mixture. Centrifuge the reaction mixture to ensure all components are at the bottom of the tube. To prevent evaporation, please overlay the sample with mineral oil if using a PCR machine without a heated lid..

*** , Note: Different DNA templates have different optimal reaction concentrations, please refer to the basic principles.**

Recommended PCR Program

Step	Temp	Time	Cycles
Pre-denaturation	98 ℃	45 s	1
Denaturation	98 ℃	10 s	
Annealing	55-65 ℃	1 min	30
Extension	72 ℃	30-60 s / kb	
Post-extension	72 ℃	1-5 min	1
Hold	4-12 ℃	∞	1

Application

1. Achieve superior amplification of bisulfite-converted

Sodium bisulfite treatment converts unmethylated cytosines to uracil, while methylated cytosines are not. In subsequent PCR amplification, thymine replaces uracil, making it possible to precisely methylate DNA to the base pair level by sequencing. The use of Gloria U 2X HS Master Mix results in high yields of bisulfite-transformed DNA with stable amplification.

2.Achieve superior amplification of damaged DNA (e.g., FFPE)

Cytosine deamination occurs on its own over a longer period of time. Deamination accelerates at elevated temperatures and leads to accumulation of uracils in DNA and free nucleotides. When other corrective enzymes are ineffective, Gloria U 2X HS Master Mix can perform high-fidelity amplification from damaged DNA templates containing uracil.

3.Prevent carryover contamination when combined with dUTP and Uracil DNA Glycosylase (UDG) treatment

Gloria U 2X HS Master Mix is easily incorporated into dUTP during amplification and can achieve 100% participation rates, so it can be combined with uracil-DNA-glycosylase (UDG) to prevent residual contamination. dUTP needs to be added to remove amplicons that may contaminate subsequent reactions by digestion of UDGs prior to amplification.

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