Superstart qPCR PreMix plus-UNG (LP)

Cat.No.:HYA422-LP

Product Description

Superstart qPCR PreMix plus-UNG (LP) is a specialized reagent specifically developed for lyophilization processes, designed for qualitative and quantitative Real Time PCR (RT-qPCR) reactions using the probe-based method. It contains Superstart Taq plus (DG), a novel hot-start enzyme with blocked Taq polymerase activity at room temperature, which effectively inhibits nonspecific amplification caused by primer nonspecific annealing or primer dimers under low-temperature conditions, thereby enhancing the specificity of amplification reactions. Formulated with an optimized qPCR-specific Buffer and a UNG/dUTP anti-contamination system, this reagent enables rapid hot start, significantly improving the amplification efficiency and detection sensitivity of qPCR reactions. It generates excellent standard curves over a wide quantitation range, ensuring accurate quantification, and effectively prevents false-positive amplification induced by PCR product carryover and aerosol contamination. This reagent is compatible with real-time PCR instruments from most manufacturers, including Applied Biosystems, Eppendorf, Bio-Rad, and Roche. After lyophilization, it exhibits excellent lyophilized morphology and product stability.

Components

1. 5×Superstart qPCR PreMix plus-UNG (Mg^{2+} free) (LP)
2. 250mM $MgCl_2$
3. 4×Lyoprotectant (Optional)

Storage

Store at -20℃ for long-term storage; stable for 3 months at 4℃. Mix thoroughly before use and avoid repeated freeze-thaw cycles.

Instructions

1. Reaction system

Components	25μL Volume	50μL Volume	Final Concentration
5×Superstart qPCR PreMix plus-UNG (Mg^{2+} free) (LP)	5 μL	10 μL	1×
250mM $MgCl_2$	0.45 μL	0.9 μL	4.5 mM
4×lyoprotectant*	6.25 μL	12.5 μL	1×
25×Primer-Probe Mix**	1 μL	2 μL	1×
Template DNA***	-	-	-
ddH_2O	Up to 25 μL	Up to 50 μL	-

*Typically, a final primer concentration of 0.2 μM yields satisfactory results; if the reaction performance is unsatisfactory, the primer concentration can be adjusted within the range of 0.2–1 μM. Generally, the probe concentration is optimized within the range of 0.1–0.3 μM. Concentration gradient experiments can be performed to identify the optimal combination of primers and probes.

**The copy number of the target gene varies among different types of templates. If necessary, serial dilution can be conducted to determine the optimal amount of template to add.

***This system is lyophilizable. If the customer has no lyophilization requirement when using this system, the 4× lyoprotectant can be added optionally; if lyophilized products are needed, the 4× lyoprotectant must be added during product performance verification at the liquid reagent stage to ensure consistency in system components and performance with the lyophilized product.

2. Reaction program

Step	Temperature	Time	Cycles
Digestion	50℃	2 min	1
Pre-denaturation	95℃	1-5 min	1
Denaturation	95 ℃	10-20 s	40-50
Annealing and Extension	56~64℃	20-60 s	

*Annealing and Extension temperature can be adjusted appropriately according to experimental requirements.

Lyophilization system preparation

Component	Volume
5×Superstart qPCR PreMix plus-UNG (Mg^{2+} free) (LP)	5 μL
250mM $MgCl_2$	0.45 μL
4×lyoprotectant	6.25 μL
25×Primer-Probe Mix	1 μL
ddH_2O	Up to 18-20 μL

Lyophilization Process

Step	Temperature	Set time	Condition	Pressure
Freezing	4℃	30 min	Hold	1 atm
	-50℃	60 min	Ramp	
	-50℃	180 min	Hold	
Primary Drying	-30℃	60 min	Ramp	Ultimate Vacuum
	-30℃	720 min	Hold	
Secondary Drying	25℃	60 min	Ramp	Ultimate Vacuum
	25℃	300 min	Hold	

This cycle protocol is suitable for in-situ lyophilization. If you need to produce lyophilized microspheres, please contact us for technical support. The parameters provided are for reference only and should be optimized to different lyophilized product formats and machines.

Guidelines for lyophilized products (in PCR tube)

1. Centrifuge the lyophilized powder instantaneously.
2. Add the template to the lyophilized powder, and add water up to 25 μL.
3. Mix and centrifuge, and then proceed with PCR amplification

Quality Control

1. Functional Testing: qPCR sensitivity, specificity, and repeatability.
2. Free of extraneous nuclease activity, as well as extraneous endonuclease and exonuclease contamination.

Technical Information

1. Superstart qPCR PreMix plus-UNG (LP) incorporates a novel hot-start enzyme, enabling rapid hot start within 1–5 minutes. Formulated with a specially optimized Buffer system, it is compatible with multiplex real-time PCR reactions.
2. It exhibits enhanced specificity and significantly improves the sensitivity for limit detection in real-time PCR. This results in marked improvements in the normalization of amplification curves and fluorescence values for ultra-low concentration templates, making it suitable for use as a high-sensitivity real-time PCR detection reagent.
3. For primers with low annealing temperatures or amplification of long fragments exceeding 200 bp, the three-step PCR protocol is recommended.
4. Different target genes vary in their dUTP utilization efficiency and sensitivity to UNG enzyme. Therefore, if the use of the UNG system leads to decreased detection sensitivity, the reaction system should be adjusted and optimized accordingly.
5. Use dedicated areas and pipettes for pre- and post-amplification steps. Operate with gloves and replace them frequently. Do not open the reaction tubes after PCR completion to minimize contamination of samples by PCR amplicons.
6. For Research Use Only !