FastAmpli RT-qPCR Premix plus-UNG

Cat.No.:HYA432

Product Description

FastAmpli RT-qPCR Premix plus-UNG is a specialized reagent for fluorescent quantitative (TaqMan probe-based) RNA amplification detection. It contains a genetically engineered and selected rapid-amplification reverse transcriptase and DNA polymerase, enabling PCR reactions to be completed within 20–40 minutes. This product exhibits strong inhibitor tolerance, enhanced reverse transcription and PCR amplification efficiency, making it suitable for high-sensitivity amplification of low-concentration RNA templates. It generates excellent standard curves over a wide quantitation range, ensuring accurate quantification. Formulated with a mixture of inhibition-resistant amplification enzyme and UNG enzyme, along with an optimized Buffer system containing dUTP, this reagent not only achieves efficient amplification of target genes in inhibitor-containing samples but also effectively prevents false-positive reactions caused by PCR product carryover and aerosol contamination. It is compatible with real-time PCR instruments from most manufacturers, including Applied Biosystems, Eppendorf, Bio-Rad, and Roche.

Components

1. 10×FastAmpli RTase/UNG Mix
2. 5×FastAmpli RT Premix Buffer (dUTP) (Mg^{2+} free)
3. 25×$MgCl_2$

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	25 uL Volume	50 uL Volume	Final Concentration
5×FastAmpli RT Premix Buffer (dUTP) (Mg^{2+} free)	5 μL	10 μL	1×
10×FastAmpli RTase/UNG Mix	2.5 μL	5 μL	1×
25×$MgCl_2$	1 μL	2 μL	1×
25×Primer-Probe Mix*	1 μL	2 μL	1×
Template RNA**	-	-	-
DEPC H_2O	Up to 25 μL	Up to 50 μL	-

*When using conventional PCR protocols for amplification, a final primer concentration of 0.2 μM typically 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.When using rapid PCR protocols for amplification, appropriately increasing the concentrations of primers and probes may yield better amplification results, and the primer-probe ratio should undergo ratio optimization.

**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.

2. Reaction program

Regular PCR program			
Step	**Temperature**	**Time**	**Cycles**
Reverse transcription	50℃	10-20 min	1
Initial denaturation	95℃	1-5 min	1
Denaturation	95 ℃	10-20 s	40-50
Annealing and Extension	56~64℃	20-60 s	

Rapid PCR program			
Step	**Temperature**	**Time**	**Cycles**
Reverse transcription	50℃	5 min	1
Initial denaturation	95℃	30 s	1

Denaturation	95 ℃	1-3 s	40-50
Annealing and Extension	56~64℃	3-20 s	

Quality Control

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

Technical Information

1. The amplification rate of the rapid DNA polymerase contained in this product is no less than 1 kb/10 s. Significant variations exist in temperature ramping rate, temperature control mode, and heat transfer efficiency among different rapid PCR instruments. It is recommended to optimize the optimal reaction parameters in conjunction with the specific rapid PCR instrument used.
2. The reverse transcription time should be optimized based on the length of the target fragments; appropriately prolonged reverse transcription time is recommended for longer fragments.
3. The annealing-extension temperature should be optimized according to the Tm values of the primers and probes, as well as the actual reaction conditions.
4. For primers with low annealing temperatures or amplification of long fragments exceeding 200 bp, the three-step PCR protocol is recommended.
5. 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. For technical support, please contact our company.
6. 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.
7. For Research Use Only !