AcuGenix™ FastAmpli qPCR Master Mix (Universal ROX)

Cat.:BR1E102

V02

BIORI BIOTECH

Product Description

AcuGenix™ FastAmpli qPCR Master Mix (Universal ROX) is a special reagent for Real-Time PCR using fluorescent dye. It contains DNA polymerase screened by gene modification and can complete PCR reaction within 30 minutes. The polymerase activity is completely blocked at room temperature by antibody modification, which can effectively inhibit non-specific amplification caused by primer non-specific annealing or primer dimer at room temperature and improve the specificity of amplification reaction. The optimized qPCR buffer greatly improves the amplification efficiency and detection sensitivity of qPCR reaction, and can obtain a good standard curve in a wide quantitative region for accurate quantification. This product contains universal ROX Reference Dye, suitable for all qPCR instruments, no need to adjust the concentration of ROX on different instruments.

Components

Components	BR1E102-41 500 T (20 μL/T)	BR1E102-42 2500 T (20 μL/T)
2×AcuGenix™ FastAmpli qPCR Master Mix (Universal ROX)*	5×1 mL	25×1 mL

*This reagent contains DNA polymerase, PCR Buffer, dNTPs, fluorescent dye, ROX Reference Dye, etc.

Storage

Store at -20±5℃, protected from light.

Notes

1.For Research Use Only. Not for use in diagnostic procedures.

2.Please prepare the reaction system in a super-clean bench. It is recommended to use a special pipette and tips with a filter element during the preparation process. Operators should wear masks and disposable gloves and change gloves frequently.

3.Mix well before use and avoid repeated freeze-thaw cycles. Repeated freeze-thawing may degrade the performance of the product. If the amount used each time is small, dispensing of the reagent is recommended.

4.Because this product contains fluorescent dye, strong light should be avoided when preparing reaction system.

5.The amplification rate of fast DNA polymerase contained in this product is no less than 1 kb/10 s. Different rapid PCR instruments have great differences in temperature rise and fall rate, temperature control mode and heat conduction efficiency. It is suggested to optimize the optimum reaction conditions in combination with the rapid PCR instruments used.

6.The reagent reaction system is specially prepared, has higher specificity, which obviously improve the sensitivity of fluorescence quantitative PCR detection, ensuring that the amplification curve normalization and fluorescence value of a very low concentration template are obviously improved, and is suitable for high-sensitivity fluorescence quantitative PCR detection.

7.For primers with low annealing temperature or long fragments over 200 bp, three-step method is recommended.

Prepare Reaction Mix

Components	Volume per Reaction
2×AcuGenix™ FastAmpli qPCR Master Mix (Universal ROX)	10 μL
Forward Primer (10 μM)[1]	0.4 μL
Reverse Primer (10 μM)	0.4 μL
Template DNA[2]	——
RNase-free ddH_2O	To 20 μL

1.When using conventional PCR procedure, the final primer concentration of 0.2 μM can get better amplification results, when the reaction performance is poor, the primer concentration can be adjusted in the range of 0.2-1 μM, when using fast PCR procedure, it is possible to get better amplification results by appropriately increasing the primer concentration.

2.Due to the different copy numbers of target gene contained in templates of different species, the template can be diluted in gradient to determine the optimal template usage.

Reaction Program

Conventional PCR procedures			
Steps	Temp	Time	Cycles
Initial denaturation[1]	95℃	1 min	1
Degeneration[2]	95℃	10 s	40
*Annealing and Elongation[3]	56-64℃	30 s	
*Melting curve analysis[4]			

Fast PCR procedures			
Steps	Temp	Time	Cycles
Initial denaturation[1]	95℃	30 s	1
Degeneration[2]	95℃	1 s	40
*Annealing and Elongation[3]	56-64℃	10 s	
*Melting curve analysis[4]			

1.The pre-denaturation conditions are applicable to most amplification reactions. The conventional PCR procedure uses 1 min, while the fast PCR procedure can be set to a minimum of 30 s. For templates with complex structures, the pre-denaturation time can be extended to 3 minutes to improve the pre-denaturation effect.
2.Denaturation: 10 s for the conventional PCR procedure; a minimum of 1 s for the fast PCR procedure.
3.Annealing/Extension: 30 s for the conventional PCR procedure. For the fast PCR procedure, the extension time can be set to 10 s for amplicons within 200 bp. For products longer than 200 bp, it is recommended to appropriately extend the extension time or adopt a three-step protocol. The annealing and extension temperature shall be adjusted according to the Tm value of the designed primers.
4.Melting curve analysis: Please set the program recommended by the fluorescence quantitative PCR instrument used.
5.* Set signal acquisition at marked position.

FAQs

1.Amplification observed in negative control?
(1)Reaction system contamination: Replace with fresh master mix, ddH_2O and primers, then repeat the experiment. Prepare the reaction system in a clean bench to reduce aerosol contamination.
(2)Primer dimer formation: Analyze in combination with the melting curve.
2.Poor linearity of the standard curve in absolute quantification?
(1) Pipette deviation: Increase the dilution fold of the template and raise the pipetting volume accordingly.
(2) Template degradation: Re-prepare the template and repeat the experiment.
(3) Excessively high template concentration: Increase the dilution fold.
3.Multiple peaks appear in the melting curve?
(1) Improper primer design: Redesign and synthesize new primers in accordance with primer design principles.
(2) Excessively high primer concentration: Reduce the primer concentration.
(3) cDNA template contaminated by genomic DNA: Prepare a new cDNA template.

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