Cat.:BR1E202

AcuGenix™ FastAmpli Probe qPCR Master Mix with UNG (Universal ROX)

V01

Product Description

AcuGenix™ FastAmpli Probe qPCR Master Mix with UNG (Universal ROX) is a specialized reagent designed for probe-based real-time PCR qualitative and quantitative analysis. It contains a genetically engineered, high-speed DNA polymerase that enables completion of PCR amplification within 30 minutes.This product is optimized with a matched buffer system suitable for multiplex amplification. This product adopts optimally formulated qPCR buffer and UNG/dUTP anti-contamination system, which greatly enhances the amplification efficiency and detection sensitivity of qPCR reaction. It can generate ideal standard curves within a wide quantification range to achieve accurate quantification, and effectively prevent false positive amplification induced by PCR product residual carryover and aerosol contamination.

Containing universal ROX Reference Dye, this premix is compatible with all qPCR instruments without the need to adjust ROX concentrations for different instruments. PCR amplification can be performed simply by adding primers and template during reaction setup.

Components

Components	BR1E202-01 100 T (20 μL/T)	BR1E202-02 500 T (20 μL/T)
2×AcuGenix™ FastAmpli Probe qPCR Master Mix with UNG (Universal ROX)*	1×1 mL	5×1 mL

*This reagent contains DNA polymerase, PCR Buffer, dNTPs, 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 ROX Reference 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, which obviously improves 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 Probe qPCR Master Mix with UNG (Universal ROX)	10 μL
20×Primer/Probe Mix[1,2]	1 μL
Template DNA[3]	——
RNase-free ddH_2O	To 20 μL

1.When amplifying with conventional PCR procedures, the optimal final primer concentration is usually 0.2 μM for good results. For poor reaction performance, the primer concentration can be adjusted within the range of 0.2-1 μM. The probe concentration is generally optimized from 0.1 to 0.3 μM. Concentration gradient experiments can be carried out to find the optimal combination of primers and probes.

2.When amplifying with fast PCR procedures, appropriately increasing the concentrations of primers and probes may achieve better amplification results, and the ratio of primers to probes should be optimized.

3.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
Digestion	50℃	2 min	1
Initial denaturation[1]	95℃	1 min	1
Degeneration[2]	95℃	10 s	40
*Annealing and Elongation[3]	56-64℃	30 s	

Fast PCR procedures			
Steps	Temp	Time	Cycles
Digestion	50℃	2 min	1
Initial denaturation[1]	95℃	30 s	1
Degeneration[2]	95℃	1 s	40
*Annealing and Elongation[3]	56-64℃	10 s	

1.Initial denaturation conditions are suitable for most amplification reactions, with standard procedures selected for 30 seconds and rapid procedures for as short as 5 seconds. If the template structure is complex, the Initial denaturation time can be extended to 3 minutes to improve the Initial denaturation effect.

2.Denaturation: 10 s for the conventional PCR procedure, and 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.* 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.