Cat.:M5144

AcuGenix™ FastAmpli RT-qPCR Master Mix with UNG V01

Product Description

AcuGenix™ FastAmpli RT-qPCR Master Mix with UNG is a specialized reagent for fluorescence quantitative (TaqMan probe-based) RNA amplification assays, containing genetically engineered reverse transcriptase and DNA polymerase optimized for rapid amplification, enabling completion of PCR reactions within 20–40 minutes. This product exhibits strong tolerance to inhibitors and demonstrates higher reverse transcription and PCR amplification efficiency, making it suitable for high-sensitivity amplification of low-concentration RNA templates. It produces reliable standard curves across a wide quantitative range for accurate quantification. The reagent employs a hybrid enzyme comprising inhibitor-resistant amplifying enzymes and UNG enzymes, along with an optimized buffer system containing dUTP, ensuring effective amplification of target genes in inhibitor-containing samples while effectively preventing false-positive reactions caused by residual PCR products or aerosol contamination. This product is compatible with fluorescence quantitative PCR systems from multiple 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) (DG)
3. 25×$MgCl_2$

Storage

Store at -20±5℃. Vortex before use. Avoid freeze-thaw cycles.

Prepare Reaction Mix

Composition	Volume per Reaction	Volume per Reaction	Final Concentration
10×FastAmpli RTase/UNG Mix	2.5 μL	5 μL	1×
5×FastAmpli RT Premix Buffer (dUTP) (Mg^{2+} free) (DG)	5 μL	10 μL	1×
25×$MgCl_2$	1 μL	2 μL	1×
25×Primer-Probe Mix [1,2]	1 μL	2 μL	1×
Template RNA [3]	——	——	——
ddH_2O	To 25 μL	To 50 μL	——

1. When using a standard PCR protocol for amplification, a final primer concentration of 0.2 μM typically yields optimal results; when reaction performance is suboptimal, the primer concentration can be adjusted within the range of 0.2–1 μM. The probe concentration is usually optimized within the range of 0.1–0.3 μM. Concentration gradient experiments can be conducted to identify the optimal combination of primers and probes.
2. When using the rapid PCR protocol for amplification, appropriately increasing the primer-probe concentration may yield better amplification results; the primer-probe ratio should be optimized accordingly.
3. Different types of templates contain varying copy numbers of the target gene; gradient dilution may be performed when necessary to determine the optimal template addition amount.

Reaction Programe

Standard PCR Program				Rapid PCR Procedure			
Procedure	Temp.	Time	Cycle	Procedure	Temp.	Time	Cycle
Digestion	50°C	2 min	1	Digestion	50°C	5 min	1
Hot Start	95°C	1-5 min	1	Hot Start	95°C	30 s	1
Denaturation	95°C	10-20 s	40-50	Denaturation	95°C	1-3 s	40-50
Annealing and Extension	56-64°C	20-60 s		Annealing and Extension	56-64°C	3-20 s	

* The temperature-sensitive TS-UNG enzyme contained in this product becomes functional at room temperature and is inactivated during the reverse transcription process.

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Quality Control

1. Functional testin: sensitivity, specificity, and reproducibility of qRT-PCR.
2. No exogenous nuclease activity, no exogenous endo/exonuclease contamination.

Notes

1. Sensi Direct qPCR Master Mix with UNG uses a new type of hot start enzyme that allows for quick activation within 1 to 5 minutes, making it particularly suitable for high-sensitivity multiplex gene detection.
2. If low fluorescence signal or significant amplification inhibition is observed, it is recommended to reduce the sample input volume or pre-dilute the sample before addition. Alternatively, the reaction volume may be increased (60-100 μL) while maintaining the same sample input volume.
3. Collection of blood, saliva, and nasopharyngeal swab samples should be performed in accordance with standard clinical operating procedures. To minimize nucleic acid degradation, freshly collected samples are recommended.
4. For primers with low annealing temperatures or amplicon fragments longer than 200 bp, it is recommended to use a three-step method for amplification.
5.The efficiency of dUTP utilization and sensitivity to UNG enzyme varies for different genes to be amplified. If the detection sensitivity is reduced due to the use of the UNG system, adjust and optimize the reaction system accordingly. If technical support is required, please contact our company.
6. Use dedicated areas and pipettes before and after amplification, wear gloves during operation, and regularly replace them. Do not open reaction tubes after PCR completion to minimize sample contamination from PCR products.