Cat.:M8254-NA

Neoscript Multi RT-qPCR Master Mix with UNG (Low DNA)

V01

Product Description

Neoscript Multi RT-qPCR Master Mix with UNG (Low DNA is a dedicated reagent for one-step qualitative and quantitative RT-qPCR using the probe method and is suitable for multiplex amplification. The entire reaction is performed sequentially in a single tube, avoiding tube opening and effectively reducing contamination. The product contains Neoscript RTase, a thermostable reverse transcriptase with reduced RNase H activity, and a genetically engineered hot-start Taq DNA polymerase, providing higher reverse transcription and PCR amplification efficiency and making it suitable for highly sensitive amplification of low-concentration RNA templates. The reagent uses an optimized qPCR buffer system together with a UNG/dUTP anti-contamination system, enabling good standard curves over a broad dynamic range for accurate quantification while effectively preventing false-positive amplification caused by residual PCR products or aerosol contamination. This reagent is compatible with most fluorescence quantitative PCR instruments from manufacturers such as Applied Biosystems, Eppendorf, Bio-Rad and Roche.

Components

1. 20×Neoscript RTase/UNG Multi Mix
2. 8×Neoscript RT Premix Multi Buffer (dUTP) (Mg^{2+} free) (DG)
3. 360 mM $MgCl_2$

Storage

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

Prepare Reaction Mix

Composition	Volume per Reaction	Volume per Reaction	Final Concentration
8×Neoscript RT Premix Multi Buffer (dUTP) (Mg^{2+} free) (DG)	3.125 μL	6.25 μL	1×
20×Neoscript RTase/UNG Multi Mix	1.25 μL	2.5 μL	1×
360mM $MgCl_2$	0.417 μL	0.833 μL	6 mM
25×Primer-Probe Mix [1]	1 μL	2 μL	1×
Template DNA [2]	——	——	——
ddH_2O	To 25 μL	To 50 μL	——

1. Usually, a final primer concentration of 0.2 μM can yield good results, when the reaction performance is poor, the primer concentration can be adjusted within the range of 0.2-1 μM. Typically, probe optimization is performed in the range of 0.1-0.3 μM. Concentration gradient experiments can be conducted to find the optimal combination of primers and probes.
2. Different types of RNA templates contain different numbers of target gene copies. If necessary, gradient dilution can be performed to determine the optimal amount of RNA template.

Reaction Programe

Standard PCR Program			
Procedure	Temp.	Time	Cycle
Reverse Transcription	50℃	10-20 min	1
Denaturation	95℃	1-5 min	1
Denaturation	95℃	10-20 s	40-50
Annealing and Extension	56-64℃	20-60 s	

*The thermolabile TS-UNG enzyme included in this product is active at room temperature and is inactivated during reverse transcription.

Quality Control

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

Zhuhai Biori Biotechnology Co.,Ltd
Tel: +86-0756-8699969 Web: www.biori-en.com Email: marketing@biori-en.com
Addr:No. 88, Shui' an 1st Road, Xiangzhou District, Zhuhai, Guangdong 519060, China

Notes

1. This system contains TS-UNG, which degrades uracil-containing DNA templates at room temperature or 25℃ and is inactivated during reverse transcription, so it does not affect subsequent PCR amplification using cDNA as the template.
2. Reverse transcription is typically performed at 50℃ in this system. The reverse transcription temperature can be optimized within the range of 42℃-55℃. According to different assay characteristics, the reverse transcription time can be optimized within the range of 5-30 min.
3. The Neoscript RTase used in this system is genetically engineered based on M-MLV, providing higher thermal tolerance and allowing a higher reverse transcription temperature. This improves reverse transcription efficiency for RNA templates with complex secondary structures.
4. This system offers good stability and broad applicability and is particularly suitable for detection of complex RNA templates such as viral RNA and RNA extracted from tissues. It provides more stable amplification of extremely low-concentration templates and is suitable for high-sensitivity molecular diagnostic reagents.
5. For primers with lower annealing temperatures or amplicons longer than 200 bp, a three-step protocol is recommended.
6. Different target genes vary in dUTP utilization efficiency and sensitivity to UNG. If the use of a UNG system reduces detection sensitivity, optimize the reaction system accordingly. Contact us for technical support.
7. Use dedicated areas and pipettes before and after amplification. Wear gloves and change them frequently. Do not open the reaction tube after PCR to minimize sample contamination by PCR products.