

Product Description

Neoscript Lyo-Ready Multi RT-qPCR Master Mix with UNG is a dedicated reagent developed for lyophilization processes, designed 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 (DG)
2. 8×Neoscript RT Premix Multi Buffer (dUTP) (Mg²⁺ free) (DG)
3. 360mM MgCl₂
4. 4×Lyoprotectant (optional)

Storage

Store at -20±5°C. 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 (DG)	1.25 µL	2.5 µL	1×
360mM MgCl ₂	0.417 µL	0.833 µL	6 mM
4×Lyoprotectant ¹	6.25 µL	12.5 µL	1×
25×Primer-Probe Mix ²	1 µL	2 µL	1×
Template DNA ³	—	—	—
ddH ₂ O	To 25 µL	To 50 µL	—

1. This system is lyophilizable. When customers use this system without the need for lyophilization, the 4×lyoprotectant may be added optionally. If lyophilized products are required, the 4×lyoprotectant must be added during product performance verification at the liquid reagent stage to ensure consistency in composition and performance with the lyophilized system.

2. 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.

3. 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 Programme

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

*The temperature-sensitive TS-UNG enzyme contained in this product can perform digestion at room temperature and become inactive during the reverse transcription process.

Prepare Reaction Mix for Lyophilization

Composition	Volume per Reaction
8×Neoscript RT Premix Multi Buffer (dUTP) (Mg ²⁺ free) (DG)	3.125 µL
20×Neoscript RTase/UNG Multi Mix (DG)	1.25 µL
360mM MgCl ₂	0.417 µL
4×Lyoprotectant	6.25 µL
25×Primer-Probe Mix	1 µL
ddH ₂ O	To 18-20 µL

*For other lyophilization system formulations, please inquire separately.

Lyophilization conditions

Procedure	Temperature	Time	status	Pressure
Pre-freezing	4℃	30 min	Hold	1 atm
	-50℃	60 min	Cooling	
	-50℃	180 min	Hold	
Primary drying	-30℃	60 min	Heating	Ultimate vacuum
	-30℃	720 min	Hold	
Secondary drying	25℃	60 min	Heating	Ultimate vacuum
	25℃	300 min	Hold	

1. This freeze-drying process is an in-situ freeze-drying process for a 25 µL system. If need to perform freeze-drying of freeze-dried beads or other in-situ systems, please inquire separately.
2. The above freeze-drying process is for reference only. Different product types and the use of different freeze dryers may result in different parameters, so adjustments can be made based on actual usage.

Instructions for Use of Lyophilized Powder

1. Centrifuge the lyophilized powder instantaneously.
2. Add the template to the lyophilized powder, and add water up to 25 µL.
3. Mix and centrifuge, and then proceed with PCR amplification.

*It is recommended to use Biori integrated mixer-centrifuge CM-8 or CM-8 Plus for standardized mixing and centrifugation operations.

Quality Control

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

Notes

1. 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.
2. 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.
3. 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.
4. For primers with lower annealing temperatures or amplicons longer than 200 bp, a three-step protocol is recommended.
5. 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.
6. 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.