NucleoGene
One Step RT-qPCR Probe Master Mix (2X)
Instructions for Use

Release Date— 01.12.2022

Store at -20°C

REF

For research use only.
Not suitable for diagnostic use.
For professional use only.

NGMM013-20 µ L x 200 rxn
NGMM013-20 µ L x 500 rxn
NGMM013-20 µ L x 1000 rxn

Description

NucleoGene One Step RT-qPCR Probe Master Mix (2X) is a convenient and highly sensitive solution for reverse transcription quantitative PCR (RT-qPCR) of RNA templates using hybridization probe detection chemistries such as TaqMan® 5 ’ -hydrolysis probes or molecular beacons on real-time quantitative PCR systems that do not require an internal reference dye. cDNA synthesis and PCR amplification are carried out in the same tube without pening between procedures. The system has been optimized todeliver maximum RT-PCR efficiency, sensitivity, and specificity, enabling unbiased co-amplification of low copy transcripts in the presence of higher copy reference genes. The proprietary reaction buffer has been specifically formulated to maximize activities of both reverse transcriptase and Taq DNA polymerase while minimizing the potential for primer dimer and other non-specific PCR artifacts. Highly specific amplification is crucial to successful qRT-PCR as non-specific products can competefor amplification of the target sequence and impair PCR efficiency. A key component of this kit is Hot Start Taq DNA polymerase,chemical modified polymerase enzyme is stable at room temperature. After a certain time after the reaction temperature rises to 95° C, the polymer polymer is activated and starts work irreversibly.

Instrument Compatibility

Different real-time PCR systems employ different strategies for the normalization of fluorescent signals and correction of well-to-well optical variations. It is important to match the appropriate reference dye to each specific optical detection system. NucleoGene One Step RT-qPCR Probe Master Mix (2X) does not contain an internal reference dye. Please check if the product is suitable for your device.

Components

NucleoGene One Step RT-qPCR Probe Master Mix (2X):
2X reaction buffer containing optimizedconcentrations of MgCl2, dNTPs (dATP, dCTP, dGTP, dTTP), hot-start DNA polymerase,reverse transcriptase, inhibitoryinactivatorsandstabilizers.

Storage and Stability

Store components in a constant temperature freezer at -25°C to -15°C protected from light upon receipt. Repeated freezing and thawing does not affect PCR performance. For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

GuidelinesforOne-Step qRT-PCR

Thaw all components, except qScript One-Step RT, at room temperature. Mix vigorously, and then centrifuge to collect contents to thebottom of the tube before using. Place all components on ice after thawing.To maximize specificity, reactions should be assembled on ice. Hotstart Taq DNA polymerase is inactive prior to high temperatureactivation; however, reverse transcriptase is active at lower temperatures. First-strand synthesis can be carried outbetween 42°C and 52°C. Optimal results are generally obtained with a 10-minute incubation at 45 – 55 ° C. We recommend a 5 minuteincubation at 95°C to fully inactivate the RT prior to PCR cycling.

Preparation of a reaction cocktail is recommended to reduce pipetting errors and maximize assay precision. Assemble the reactioncocktail with all required components except RNA template and dispense equal aliquots into each reaction tube. Add RNA to eachreaction as the final step. Addition of sample as 5 to 10-µL volumes will improve assay precision.

Suggested input quantities of template are: 1 pg to 1 µg total RNA; 10 fg to 100 ng poly A(+) RNA; 10 to 1x10^{8}copies viral RNA. After sealing each reaction, vortex gently to mix contents. Centrifuge briefly to collect components at the bottom of the reaction tube.

Component	Volume for 20-µL rxn.	Final Concentration
One-Step Master Mix (2X)	10 µl	1X
Forward primer	Variable	400 – 900 nM
Reverse primer	Variable	400 – 900 nM
Probe	Variable	50-200 nM
Nuclease-free water	Variable	
RNA template	1- 5 µL	Variable
Enzyme Mix(40X)	0,5 µL	1X
Final Volume (µL)	**20 µL**	

Real Time PCR Protocol

	Standard Cycling
cDNA Synthesis (1 cycle)	50° C, 15 min
Initialdenaturation (1 cycle)	95ºC 2 min 30 sec
Denaturation Annealing/Extension (35-45 cycles)	95ºC 15s ***55-60ºC 30s**

THA appropriate step for fluorescent data collection varies for different probe types. Data collection for the hydrolysis probe assays (TaqMan probe) should be performed at theend of the extension step. Use the annealing step for data collection in hybridization probe experiments (HybProbe® FRET hybridization probes, Molecular Beacons, Solaris® qPCR Assays, Scorpions® primers, etc.). Detection probe chemistry endpoint analysis should be performed at an appropriate temperature. The optimal initial denaturation time of the DNA polymerase depends on the DNA template and affects the qPCR efficiency and sensitivity. Reproduction of genomic DNA orplasmid DNA targets may take 10 minutes at 95° C to completely denature and fragment the template. THA single stranded DNA template, such as the short doublestranded DNA

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template (PCR product) orcDNA, may require as little time as 1 to 30 seconds at 95 ° C.

THA extension time depends on the amplicon length and the minimum data collection time requirement for your qPCR. As a general starting point, you can use 30 seconds at 60 ° C. For any primer set and Real Time PCR device, the optimum annealing temperature and duration may need to be specifically determined.

Quality Control

Kit components are free of contaminating DNase and RNase. NucleoGene One Step RT-qPCR Probe Master Mix (2X) is functionally tested in qPCR. Kinetic analysis must demonstrate linear resolution over six orders of dynamicrange (R2 > 0.990) with a 2-fold discrimination of starting template and a PCR efficiency>95%.

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