NucleoGene
One Step RT-qPCR
Sybr Green Master Mix
Instructions for Use

Release Date— 01.12.2019

Store at -20°C

REF

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

NGMM016 20- µ L x 200 rxn
20- µ L x 500 rxn
20- µ L x 1000 rxn

Description

NucleoGene One Step RT-qPCR Sybr Green Master Mix (2X) is a convenient and highly sensitive solution for reverse transcription quantitative PCR (RT-qPCR) of RNA templates using SYBR Green I dye detection and gene-specific primers. cDNA synthesis and PCR amplification are carried out in the same tube without opening between procedures. The system has been optimized to deliver maximum RT-PCR efficiency, sensitivity, and specificity. 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 nonspecific PCR artifacts. The kit is compatible with both fast and standard qPCR cycling protocols. Highly specific amplification is essential for successful RT-qPCR with SYBR Green I technology, since this dye binds to any dsDNA generated during amplification. 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 Sybr Green 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 Sybr Green Master Mix Reaction Mix (2X): 2X reaction buffer containing optimized concentrations of MgCl2, dNTPs (dATP, dCTP, dGTP, dTTP), hot-start DNA polymerase, reverse transcriptase, SybrGreen Dye, inhibitory inactivators and stabilizers.

Storage and Stability

Store components in a constant temperature freezer at -25° C to -15° C protected from light up on 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.

Guidelines for One-Step Sybr Green qRT-PCR

Primer design is critical for successful one-step RT-qPCR with SYBR Green. The use of software tools for PCR primer design and RNA secondary structure analysis can aide in the design of specific and efficient primers for one-step RT-PCR. Primers should be designed according to standard qPCR guidelines with a length of 18- 25 nucleotides and a GC content of 40-65%. Avoid internal secondary structure, and complementation at 3' ends within each primer and primer pair. 3'-end terminal stability should be kept low to maximize primer specificity (3'-pentamer ΔG° > -8.0 kcal/mol or have no more than 2 to 3 Cs or Gs in the last 5 bases). Regions of RNA secondary structure should be avoided as this can interfere with annealing of the reverse primer for cDNA synthesis and/or impede procession of the reverse transcriptase.

Ideally, primer Tm should be between 58 and 60°C for a typical 2-step qPCR cycling protocol. Estimation of primer Tm varies widely with different methods and analysis parameters. We recommend using a program that calculates Tm based on nearest-neighbor thermodynamic models at 50 mM monovalent salt and 50 nM primer concentration. Primers with melting temperatures outside of this range may require optimization of PCR cycling conditions. PCR product size should be between 70- 200 bp. Ideally, the amplified sequence should span intronic sequence to minimize the potential to amplify genomic DNA sequence. Control reactions that lack reverse transcriptase (minus RT) should always be included to verify that amplification signal is due to the presence of RNA target and not genomic DNA. A final concentration of 200 nM each primer is recommended as a general starting point. Optimal results may require titration of primer concentration between 100 and 500 nM. PCR efficiency is often improved with higher primer concentration (300 to 500 nM). In some cases, higher concentration of the reverse primer alone may improve RT-PCR efficiency without compromising specificity. We highly recommend including a post PCR dissociation analysis step (melt curve) to distinguish specific from non-specific amplification product(s) (i.e. primer-dimer). Thaw all components, except NucleoGene One Step RT-qPCR Sybr Green Master Mix (2X), at room temperature. Mix by gently vortexing, then centrifuge to collect contents to the bottom of the tube before using. Place all components on ice after thawing. To maximize assay specificity and sensitivity reactions should be assembled on ice and kept cold until placed in your real-time PCR system. Centrifugation steps should be carried out in a refrigerated centrifuge. Hotstart Taq DNA polymerase is inactive prior to high temperature activation; however, reverse transcriptases are active at lower temperatures and can use single strand DNA as a template. First-strand synthesis can be carried out between 42° C and 52° C. Optimal results are generally obtained with a 5-minute incubation at 50° C. We recommend a 10-15 minute incubation 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 reaction cocktail with all required components except RNA template and dispense equal aliquots into each reaction tube. Add RNA to each reaction 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 100 ng 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
Reaction Mix (2X)	10 µl	1X
Enzyme Mix (40X)	0.5 µl	1X
Forward primer	Variable	200 – 400 nM
Reverse primer	Variable	200 – 400 nM
Nuclease-free water	Variable	
RNA template	5 – 10 µL	Variable
Final Volume (µL)	**20 µL**	

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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**
Melt curve analysis	follow instrument recommendations

***In the temperature cycles written in bold, take readings from the Sybr Green channel.**

RT step at 50 ° C is optimal for Reverse Transcriptase, however thereaction can be performed at temperatures 45‑60 ° C. For difficult templates with high secondary structure, the temperature may be increased to 60 ° C.

IMPORTANT: Enzyme activation step at 95 ° C for 2 min 30 sec is crucial for full activation of DNA polymerase and inactivation of reverse transcriptase.

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

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