NucleoGene cDNA Synthesis Super Mix Kit (2X)

Instructions for Use

Release Date— 01.12.2023

Store at -20°C

REF

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

NGMM032 25 x 20 µl rxn
50 x 20 µl rxn
100 x 20 µl rxn
200 x 20 µl rxn

Description

Nucleogene cDNA Synthesis Super Mix Kit (2X) provides a sensitive and easy-to-use solution for two-step RT-PCR. This 5X concentrated master mix provides all necessary components (except RNA template) for first-strand synthesis including: buffer, dNTPs, MgCl2, primers and stabilizers. qScript is a RNase H(+) derivative of MMLV reverse transcriptase, optimized for reliable cDNA synthesis over a wide dynamic range of input RNA. The unique blend of oligo (dT) and random primers in the Nucleogene cDNA Synthesis Super Mix Kit (2X) works exceptionally well with a wide variety of targets. This blend is optimized for the production of targets < 1kb in length. Nucleogene cDNA Synthesis Super Mix Kit (2X) produces excellent results in both real-time and conventional RT-PCR.

Composition

Nucleogene cDNA Synthesis Super Mix Kit (2X) reaction buffer containing optimized concentrations of MgCl2, dNTPs (dATP, dCTP, dGTP, dTTP) and stabilizers. Enzyme Mix containing recombinant RNase inhibitor protein, qScript reverse transcriptase.

Storage and Stability

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

Reaction Assembly

Place components on ice. Mix, and then briefly centrifuge to collect contents to the bottom of the tube before using.

Component	Volume for 20-µL rxn.	Final Concentration
NucleoGene cDNA Reaction Mix (2X)	10 µL	1X
NucleoGene cDNA Enzyme Mix (20X)	1 µL	1X
RNA template	Variable	(1 µg to 10 pg total RNA)
RNase/DNase-free water	Variable	
Total Volume (µL)	20 µL	

Reaction Protocol

• Combine reagents in 0.2-mL micro-tubes or 96-well plate sitting on ice.
• After sealing each reaction, vortex gently to mix contents. Centrifuge briefly to collect components at the bottom of the reaction tube.

Incubate:
5 minutes at 25° C
30 minutes at 50° C
5 minutes at 85° C
Hold at 4° C

• After completion of cDNA synthesis, use 1/5th to 1/10th of the first-strand reaction (2-4 µL) for PCR amplification. If desired, cDNA product can be diluted with 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA and stored at -20° C.

Guidelines for Reverse Transcription-qPCR

Minus RT-controls: Accurate quantification of gene expression by RT qPCR requires testing and reporting the extent of contamination of genomic DNA in each RNA sample for each gene of interest. The presence of trace amounts of gDNA does not usually interfere with quantification of high copy reference genes. However, it can have a significant contribution on signal for low copy genes. Even when using primers that are separated by intronic sequence or bridge exon junctions, the presence of genomic DNA can produce positive signals from amplification of pseudogene or off-target PCR product. Therefore, it is important to always include the appropriate “no RT” or “minus RT” control reactions in your experimental design. Since the reverse transcriptase is an integral component of Nucleogene cDNA Synthesis Super Mix Kit (2X), it is not feasible to construct a formal cDNA synthesis control that includes all components except the RT. The most direct method to test for the presence of genomic DNA is to bypass the RT step and use an equivalent amount of the RNA preparation directly for PCR amplification. For example: if you start with 1 µg of total RNA for cDNA synthesis and use 1/10th of the first-strand reaction as template for qPCR; then use 100 ng of total RNA as template for the minus RT-control qPCR. Any signal from the RNA only reaction is attributable to the presence of genomic DNA. DNase digestion of total RNA: Trace levels of genomic DNA can obscure accurate quantification, particularly when the specific gene(s) of interest are low copy. Nucleogene DNase I is a high purity, recombinant DNase I preparation that is free of any contaminating RNases. It provides a simple and rapid solution to eliminate residual genomic DNA that is directly compatible with Nucleogene cDNA Synthesis Super Mix Kit (2X), or other first-strand synthesis kits. The supplied Reaction Buffer and proprietary Stop Buffer support a simple heat-kill step that permanently inactivates all trace levels of DNase activity before the cDNA synthesis step. Heat-kill procedures used by other DNase I reagents are ineffective and not compatible with Nucleogene cDNA Synthesis Super Mix Kit (2X). Residual, or renatured, DNase will degrade cDNA product and alter apparent expression levels. If using other sources of RNase-free DNase I, it is essential to remove all traces of DNase activity before proceeding with first-strand synthesis. Suitable RNA purification methods include phenol:chloroform extraction followed by ethanol precipitation, or the use of chaotropic salts and a silica-based RNA purification column. Please visit our web site at **www.nucleogene.com** if you require additional information or protocols.

Quality Control

Kit components are free of contaminating DNase and RNase. Nucleogene cDNA Synthesis Super Mix Kit (2X) is functionally tested in reverse transcription quantitative PCR (RT-qPCR). First-strand synthesis is performed in triplicate on each dilution of a log-fold serial dilution of HeLa cell total RNA from 1 pg to 1 µg. One-tenth of each first-strand reaction is used for qPCR amplification. Kinetic analysis must demonstrate linear resolution over five orders of dynamic range (r2 > 0.995) and a PCR efficiency > 90%.

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