NucleoGene microRNA cDNA Synthesis Kit

Instructions for Use

Release Date— 01.01.2022

Store at -20°C

REF

NGMM022 25 x 20 µl rxn
100 x 20 µl rxn

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

Description

NucleoGene microRNA cDNA Synthesis Kit is an all-in-one, ready-to-use product for the reverse transcription of microRNA from either Total RNA preparations or enriched microRNA preparations. The kit contains the 2x Reaction Mix and the microScript microRNA Enzyme Mix. The kit utilizes NucleoGene Reverse Transcriptase, a mutant version of Moloney Murine Leukemia Virus (M-MuLV) Reverse Transcriptase. It has reduced RNase H activity and increased thermal stability. The workflow of NucleoGene microRNA cDNA Synthesis Kit involves a simple, singletube set-up by the mixing of 2x Reaction Mix, Enzyme Mix and the RNA template. The reaction can then be carried out in a thermocycler. A poly (A) tail is first added to the RNA template, followed by cDNA synthesis using an adapter primer. In addition to the ease-of-use, the singletube set-up provides superb consistency and sensitivity. The cDNA could be used in a PCR or qPCR amplification using a Universal PCR Reverse Primer and the forward primer that contains the sequence of the microRNA of interest. A single cDNA preparation could be used for PCR amplification of a number of different microRNAs. In addition, the cDNA preparation could be used for PCR or qPCR detection (using gene-specific forward and reverse primers) of mRNA or large RNA if Total RNA preparation was the starting template. This could allow for parallel evaluation of expression levels of microRNAs and microRNA-targets.

Composition

NucleoGene microRNA cDNA Synthesis Kit containing of; Poly(A) Polymerase, 2X Reaction Buffer, microRNA Enzyme Mix, dNTPs (dATP, dCTP, dGTP,dTTP), recombinant RNase inhibitor protein, qScript reverse transcriptase and stabilizers.

Storage and Stability

Store components in a constant temperature freezer at -25°C to -15°C upon receipt.For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

Reaction Protocol

1. Thaw template RNA and all reagents on ice. Mix each solution by vortexing gently, and centrifuge briefly to collect residual liquid from the sides of the tubes.

2. Prepare the following reaction mixture in a tube on ice. Mix gently and centrifuge briefly to collect contents:

Component	Volume for 20-µL rxn.
RNA template (1 pg to 1 ug total RNA or enriched microRNA)	X µL
2x Reaction Mix	10 µL
microRNA Enzyme Mix	1 µL
RNase/DNase-free water	X µL
Total Volume (µL)	**20 µL**

The procedure could be used for 1 pg to 1 µg of total RNA or enriched microRNA. It is highly recommended that RNA is isolated by methods that recover microRNAs. Nucleogene provides an extensive line of RNA extraction products that recover all sizes of RNA including microRNAs, without the use of inhibitory chemicals such as phenols. A list of recommended kits are provided at the end of this insert or can be found at

Reaction Protocol First-Strand microRNA cDNA Synthesis

Temperature	Time
37°C	30 minutes
50°C	30 minutes
95°C	5 minutes
4 °C	Hold

Notes

- RNA samples must be free of genomic DNA contamination.
- Multiple freezing and thawing of RNA should beavoided.
- The synthesized first-strand miRNA should be stored at -20 ° C.

Procedure for PCR Amplification of microRNA cDNA Synthesized

The cDNA generated can now be used as a template in a PCR reaction. In general, dilute the cDNA 2 - 5 fold using nuclease-free water. Use 1 - 8 uL of the diluted cDNA in a 20 uL PCR reaction (such as with NucleoGene 2x Hot Start PCR Master Mix, Cat.# NGMM001). For quantitative PCR, spike in an appropriate amount of SYBR Green I. The PCR reaction should be set up with the Universal PCR Reverse Primer (provided) and a microRNA-specific forward primer, as indicated in Table 1 below.

TABLE 1.

Components	Volume per Reaction
2x Hot Start PCR Master Mix (with or without SYBR Green I)	**10 ul**
microRNA-specific Forward Primer (5 uM)	**1 ul**
Universal PCR Reverse Primer	**1 ul**
microRNA cDNA (recommended to be diluted 2 - 5 fold)	**X ul**
Nuclease-Free Water	**X ul**
Total Volume	**20 ul**

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Reaction Protocol microRNA PCR

TABLE 2.

Temperature	Time	Cycles
95ºC	15 min	1
95ºC	15 sec	40
60ºC	30 sec	
72ºC	45 sec	

The cDNA generated can also be used as a template in a PCR reaction for other RNA transcripts (such as mRNA and other large RNA). In general, dilute the cDNA 2 - 5 fold using nuclease-free water. Use 1 - 8 uL of the diluted cDNA in a 20 uL PCR reaction (such as with NucleoGene 2x Hot Start PCR Master Mix, Cat.# NGMM001). For quantitative PCR, spike in an appropriate amount of SYBR Green I. The PCR reaction should be set up with the genespecific primers (not provided), as indicated in Table 3 below. The PCR amplification could be performed according to Table 2 above or according to the Gene-specific primer thermal properties.

TABLE 3.

PCR Reaction Set-up for mRNA or Large RNA

Components	**Volume per Reaction**
2x Hot Start PCR Master Mix (with or without SYBR Green I)	**10 ul**
Gene-specific Forward Primer (5 uM)	**1 ul**
Gene-specific Forward Primer (5 uM)	**1 ul**
cDNA (recommended to be diluted 2 - 5 fold)	**X ul**
Nuclease-Free Water	**X ul**
Total Volume	**20 ul**

Quality Control

Kit components are free of contaminating DNase and RNase. Nucleo-genemi miRNA to cDNA Mix is functionally tested in reverse transcription quantitative PCR (RT-qPCR). First-strand synthesis is performed in tripli-cate on each dilution of a log-fold serial dilution of HeLa cell total RNA from 1 pg to1 µg. One-tenth of each first-strand reaction is used for qPCR amplification. Kinetic analysis must demonstrate linearresolution over five orders of dynamic range (r2> 0.995) and a PCR efficiency > 90%.

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