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microScript microRNA cDNA Synthesis Kit Product Insert

Norgen's microScript 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 Norgen's TruScript 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 Norgen's microScript microRNA cDNA Synthesis Kit involves a simple, single-tube 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 single-tube 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.

Kit Components

Component	Product # 54415 (12 Reactions)	Product # 54410 (50 Reactions)
microScript microRNA Enzyme Mix	12 µL	50 µL
2x Reaction Mix	120 µL	500 µL
Universal PCR Reverse Primer	60 µL	250 µL
Nuclease-Free Water	1.25 mL	2 x 1.25 mL
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Storage Conditions and Product Stability

Norgen's microScript microRNA cDNA Synthesis Kit components should be stored at -20°C. These reagents should remain stable for at least 1 year in their unopened containers.

Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use. Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

Procedure for First-Strand microRNA cDNA Synthesis

Materials to be supplied by user

- Thermocycler
- Nuclease-Free PCR Tubes compatible with Thermocycler
- Ice

1. 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. Norgen Biotek 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 *www.norgenbiotek.com*.

   Note: Higher amounts (>1 µg) of RNA input can be used, however it is highly recommended that the volume of the reaction be scaled up also.

2. Thaw the RNA template, Nuclease-Free Water and 2x Reaction Mix on ice. The microScript microRNA Enzyme Mix should be kept at -20°C at all times until just before adding to the reaction mix, and should be returned to -20°C immediately.

3. Set up the First-Strand microRNA cDNA Synthesis reaction in a tube compatible with the thermocycler to be used, as described in **Table 1**.

Table 1. First-Strand microRNA cDNA Synthesis Reaction Set-up

Component	Volume per Reaction
2x Reaction Mix	10 µL
microScript microRNA Enzyme Mix	1 µL
RNA template (1 pg to 1 µg total RNA or enriched microRNA)	x µL
Nuclease-Free Water	x µL
Total Volume	**20 µL**

4. Incubate First-Strand microRNA cDNA Synthesis reaction in a thermocycler as described in **Table 2**.

Table 2. Reaction Protocol First-Strand microRNA cDNA Synthesis

Temperature	Time
37°C	30 minutes
50°C	30 minutes
70°C	15 minutes
4°C	Hold

5. 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. Un-used cDNA should be stored at -20°C.

Suggested Procedure for PCR Amplification of microRNA cDNA Synthesized

Additional Materials to be supplied by user

- PCR or qPCR Master Mix (such as with Norgen's 2x PCR Master Mix, Cat.# 28007)
- microRNA-specific Forward Primer, 5 μM (validated primers are available from Norgen Biotek Corp.)

1. 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 μL of the diluted cDNA in a 20 μL PCR reaction (such as with Norgen's 2x PCR Master Mix, Cat.# 28007). 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 3** below.

Table 3. Suggested PCR Reaction Set-up for microRNA

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

Note: Validated microRNA-specific Forward Primers are available from Norgen Biotek. Alternatively, a user-provided primer could be used. To design a microRNA-specific forward primer, either the whole sequence of the microRNA of interest could be used or it could be designed with a primer-design program with an annealing temperature setting of 60^{o}C.

2. Perform the PCR amplification in a thermocycler as described in **Table 4**.

Table 4. Reaction Protocol microRNA PCR

Temperature	Time	Cycles
94^{o}C	3 minutes	1 Cycle
94^{o}C	15 seconds	40 Cycles
60^{o}C	30 seconds	
72^{o}C	45 seconds*	

* Collect fluorescent data for qPCR

3. 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 μL of the diluted cDNA in a 20 μL PCR reaction (such as with Norgen's 2x PCR Master Mix, Cat.# 28007). For quantitative PCR, spike in an appropriate amount of SYBR Green I. The PCR reaction should be set up with the gene-specific primers (not provided), as indicated in **Table 5** below. The PCR amplification could be performed according to **Table 4** above or according to the Gene-specific primer thermal properties.

Table 5. Suggested PCR Reaction Set-up for mRNA or Large RNA

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

Related Products	Product #
Total RNA Purification Kit	17200, 37500
Total RNA Purification Plus Kit	48300, 48400
Total RNA Purification Micro Kit	35200
microRNA Purification Kit	21300
All-in-One Purification Kit	24200, 24210
RNA/Protein Purification Kit	23000
RNA/DNA/Protein Purification Kit	24000
RNA/DNA/Protein Purification Plus Kit	47700
RNA/DNA Purification Kit	48700
FFPE RNA Purification Kit	25300
FFPE RNA/DNA Purification Plus Kit	54300
Urine microRNA Purification Kit	29000
Urine Exosome RNA Isolation Kit	47200
Plasma/Serum RNA Purification Kit	55000
Plant/Fungi Total RNA Purification Kit	25800

Technical Support

Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362. Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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