



		<b>miRNA EasyScript™ cDNA Synthesis Kit</b>	
<b>Part No.</b>	<b>Cat. No.</b>	<b>G269</b>	<b>G270</b>
RT-1	EasyScript™ RTase (200 U/μl)	25 μl	100 μl
RT-5	dNTPs (10 mM)	40 μl	160 μl
RT-6	RNaseOFF Ribonuclease Inhibitor (40 U/μl)	15 μl	60 μl
RT-7	5X RT buffer	150 μl	600 μl
RT-9	miRNA Oligo (dT) adapter (10 μM)	60 μl	240 μl
RT-0	RNase-free H <sub>2</sub> O	1 ml	2 x1 ml
	Size	25 rxns	100 rxns

## Product Description

**EasyScript™ Reverse Transcriptase** is an optimized mutational derivative of the original RTase enzyme representing the best-performing RTase on the market. This enzyme catalyzes the synthesis of complementary DNA strands from single-stranded RNA or DNA templates. Due to a series of mutations introduced within the RNase H domain of this enzyme, there is no detectable RNase H activity associated with the enzyme. The lack of RNase H activity helps to eliminate RNA degradation during first-strand cDNA synthesis, resulting in better yield and length of cDNA synthesized. Furthermore, EasyScript™ RTase contains an additional fidelity-enhancing subunit which drastically enhances accuracy in reverse transcription.

**miRNA EasyScript™ cDNA Synthesis Kit** is a complete system for the efficient synthesis of first strand miRNA from total RNA templates. The recombinant RNaseOFF Ribonuclease Inhibitor, supplied with the kit effectively protects RNA template from degradation. The kit includes an unique miRNA Oligo d(T) adapter that anneals to the Poly (A) tail of miRNA template, thus facilitating easy cDNA synthesis. Furthermore, the miRNA Oligo d(T) adapter enables the use of our universal primer in the miRNA profiling kit (MA003 or MA004, abm), eliminating the need for additional and costly miRNA-specific primers. The synthesized cDNA can be directly used as a template for the qPCR-based analysis of miRNA expression.

## Storage Buffer

20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01 % (v/v) NP-40, 50 % (v/v) glycerol.

## Storage Condition

Store all components at -20 °C in a non-frost-free freezer. All components are stable for 1 year from the date of shipping when stored and handled properly.



## Protocol

Reverse transcription reactions should be assembled in a RNase-free environment. The use of “clean”, automatic pipettes designated for PCR and aerosol-resistant barrier tips are recommended.

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:

**Note:** Template RNA must contain Poly (A) tail for the annealing of miRNA Oligo (dT) adapter. Obtain Poly (A) + RNA template using Poly (A) tailing reaction kit (Cat.No. :AM1350, Ambion).

	Volume	Final Concentration
Poly(A) + RNA template	10 $\mu$ l	Variable (~2 $\mu$ g total RNA or 200 ng small RNA)
miRNA Oligo (dT) adapter (10 $\mu$ M)	2 $\mu$ l	1 $\mu$ M

3. Incubate at 65 °C for 5 minutes to allow annealing of miRNA Oligo (dT) adapter to Poly (A) + RNA template. Quickly chill on ice and centrifuge briefly to collect contents. Add the following components:

	Volume	Final Concentration
dNTPs (10 mM)	1 $\mu$ l	500 $\mu$ M
5X RT buffer	4 $\mu$ l	1X
RNaseOFF Ribonuclease Inhibitor (40 U/ $\mu$ l)	0.5 $\mu$ l	20 U/rxn
EasyScript™ RTase (200 U/ $\mu$ l)	1 $\mu$ l	200 U/rxn
RNase-free H <sub>2</sub> O	1.5 $\mu$ l	-
Final Volume	20 $\mu$ l	-

4. Mix well by finger-vortex and centrifuge briefly. Incubate at 42°C for 15 minutes.

5. Immediately stop the reaction by heating at 70 °C for 10 minutes. Chill on ice. The newly synthesized first-strand of miRNA can be directly used as a template for the qPCR-based analysis of miRNA expression.

## Notes

1. RNA samples must be free of genomic DNA contamination.
2. Multiple freezing and thawing of RNA should be avoided.
3. All reagents should be kept cold for longer shelf-life.
4. The synthesized first-strand miRNA should be stored at -20 °C.
5. Five microliters of the final product is necessary for one 384-well miRNA Profiling Array (MA003 or MA004, abm)