

# miRcute miRNA First-strand cDNA Synthesis Kit

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For first-strand cDNA synthesis from miRNA



# miRcute miRNA First-strand cDNA Synthesis Kit

Cat. no. KR201

## Kit Contents

Contents	KR201-01 25 preps	KR201-02 50 preps
<i>E.coli</i> Poly (A) Polymerase (5 U/μl)	14 μl	28 μl
10x Poly (A) Polymerase Buffer	60 μl	120 μl
5x rATP Solution	120 μl	240 μl
10x RT Primer	60 μl	120 μl
10x RT Buffer	70 μl	150μl
Super pure dNTP (2.5 mM each)	30 μl	60 μl
RNasin (40 U/μl)	30 μl	60 μl
Quant RTase	15 μl	30 μl
RNase-free ddH <sub>2</sub> O	1 ml	1 ml
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## Storage

miRcute miRNA First-strand cDNA Synthesis Kit should be stored at -20 °C.

## Introduction

miRcute miRNA First-strand cDNA Synthesis Kit adds Poly(A) to the 3'-terminal of miRNA and synthesizes the first-strand cDNA based on Poly(A) modified miRNA through oligo(dT)-universal tag primed reverse transcription.

The kit contains all the necessary reagents in the process of adding Poly(A) and reverse transcription. The kit can provide high efficient Poly(A) modification and reverse transcription and efficiently synthesize the first-strand cDNA from 20 pg-2 µg total RNA.

**Note: This kit has to be used with miRcute miRNA qPCR Detection Kit (SYBR Green) (Cat. no. FP401).**

### Important Notes

1. Change the disposable gloves frequently to avoid the RNase contaminations from skin.
2. Use RNase-free plastic wares and tips to avoid cross-contaminations.

### Protocol

1. Add Poly (A) to the 3' terminal of miRNA
- 1) Prepare a reaction solution in a pre-cooling tube on ice according to the following table (Add *E.coli* Poly (A) Polymerase in the last step).

Contents	Volume/ Reaction	Final concentration
Total RNA*	-	≤2 µg
<i>E.coli</i> Poly (A) Polymerase (5 U/µl)	0.4 µl	2 U
10× Poly (A) Polymerase Buffer	2 µl	1×
5× rATP Solution	4 µl	1×
RNase-free H <sub>2</sub> O	-	-
Total volume	20 µl	

**\*Total RNA as the template has to contain miRNA in the reaction. miRNA can also be used as template in the reaction (2-5µl miRNA is recommended. Determine the amount according to the abundance of the target miRNA.)**

- 2) Mix gently by pipetting the solution and centrifuge briefly to remove the drops from walls of tube. Incubate at 37°C for 60 min. The solution can be used directly to the downstream experiments or stored at -20 °C for a short time. For long storage, -80°C is recommended.

2. Reverse transcription of Poly (A) modified miRNA

- 1) Prepare a reaction solution according to the following table

Contents	Volume
Poly (A) reaction solution	2 $\mu$ l
10x RT Primer	2 $\mu$ l
10x RT Buffer	2 $\mu$ l
Super pure dNTP (2.5 mM each)	1 $\mu$ l
RNasin (40 U/ $\mu$ l)	1 $\mu$ l
Quant RTase	0.5 $\mu$ l
RNase-free ddH <sub>2</sub> O	11.5 $\mu$ l
Total volume	20 $\mu$ l

- 2) Mix gently by pipetting the reaction solution and briefly centrifuge to remove drops from the wall of the tube. Incubate at 37°C for 60 min.

The reaction solution including cDNA products can be stored at -20°C or used in downstream quantitative PCR directly.

## Ordering Information

### RNA Isolation

Product	Size	Cat.no.
miRcute micro RNA Isolation Kit	50 preps	DP501

### Real-Time PCR

Product	Size	Cat.no.
miRcute miRNA qPCR Detection Kit (SYBR Green)	50 $\mu$ l $\times$ 50 rxns	DP401