miRcute Plus miRNA First-Strand cDNA Kit

For first-strand cDNA synthesis from miRNA

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miRcute Plus miRNA First-Strand cDNA Kit

Cat. No. GKR211

Kit Contents

Contents	GKR211-01 (25 rxn)	GKR211-02 (50 rxn)
miRNA RT Enzyme Mix	50 μl	100 μl
2 × miRNA RT Reaction Buffer	250 μl	500 μl
RNase-Free ddH_2O	1 ml	1 ml
Handbook	1	1

Storage

Upon receiving this product, please immediately store it at -30~-15°C. When it is taken out from -30~-15°C, keep the miRNA RT enzyme mix on ice. Thaw the 2× miRNA RT reaction buffer and mix it upside down slightly after thawing. The solution should be used when it's completely uniform.

The product can be stored for 1 year at -30~-15°C.

Introduction

miRcute Plus miRNA First-strand cDNA 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.

miRcute Plus miRNA First-strand cDNA Kit has only two components, which simplifies the operation procedure and reduces the possibility of operation error. miRNA RT Enzyme Mix in this product contains the *E.coli* Poly(A) Polymerase, Reverse transcriptase and RNase Inhibitor. The *E.coli* Poly(A) Polymerase not only has higher polymerase efficiency and A tailing efficiency, but also can specifically recognize single strand miRNA, avoiding the further reverse transcription reaction of microRNA precursors which has double strand structures. Reverse transcriptase has been modified at the molecule level to remove the activity of RNase H, which increases the affinity of RNA template, making the reverse transcriptional reaction of miRNA a higher efficiency and sensitivity.

The 2× miRNA RT Reaction Buffer contains all the materials and primers used in miRNAs A-tailing and reverse transcription, which could guarantee the A-tailing and reverse transcription reaction of miRNAs are carried on at the same time and make the process more efficiently.

Note: This kit has to be used with miRcute Plus miRNA qPCR Kit (SYBR Green) (Cat. no. GFP411-01/GFP411-02).

Kit Highlights

1. Combined the A-tailing reaction and reverse transcription in one tube, which will save operation time and reduce operation steps.
2. The *E.coli* Poly(A) Polymerase not only has higher polymerase efficiency and A tailing efficiency, but also can specifically recognize single strand miRNA, avoiding the further reverse transcription reaction of microRNA precursors which has double strand structures.
3. The detection rate of low abundance miRNA is greatly improved by simplified operation procedure.
4. This kit can be used for reverse transcriptional reaction of miRNA extracted from almost all materials. The dosage range of the templates can reach 20 pg-2 μg (mass range) and 10 fM-100 pM (concentration range).

Protocol

1. Fully thaw RNA template, 2× miRNA RT Reaction Buffer and RNase-Free ddH_2O. Centrifuge transiently and put all of them on ice.
2. Prepare a reaction solution according to the following table (all the steps should be operated on ice).

Contents	20 μl Volume /Reaction	Final concentration
Total RNA*	-	≤2 μg
2× miRNA RT Reaction Buffer	10 μl	1×
miRNA RT Enzyme Mix	2 μl	1×
RNase-Free ddH_2O	-	-
Total volume	20 μl	

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

3. Mix gently by pipetting the reaction solution and briefly centrifuge to remove drops from the wall of the tube. Incubate at 42°C for 60 min and then 95°C for 3 min. The details are shown in the following table:

Temperature	Reaction time	Contents
42°C	60 min	A-tailing and RT
95°C	3 min	Enzyme inactivation

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

Important Notes

1. Always replace to new gloves. Because the skin often contains bacteria, which may cause RNase pollution.
2. Use RNase-Free plastic products and tips to avoid cross-contamination.
3. RNA will not be degraded by RNase in the lysate. However, RNase-Free plastic and glassware should be used for the following operations after extraction. Bake the glassware at 150°C for 4 hours, or soak the plastic ware in 0.5 M NaOH for 10 minutes, then wash thoroughly with RNase-Free ddH_2O, and then subject to autoclave to remove RNase.
4. Prepare the solution with RNase-Free ddH_2O (add the ddH_2O into a clean glass bottle, add DEPC to the final concentration of 0.1% (V/V), incubate overnight, then autoclave).