InRcute lncRNA First-Strand cDNA Kit

Ultra-sensitive lncRNA specific reverse transcription reagent

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lnRcute lncRNA First-Strand cDNA Kit

Cat. no. GKR202

Kit Contents

Contents	GKR202-01 (25 rxn)	GKR202-02 (100 rxn)
5×gDNA Buffer	50 µl	200 µl
lnR-RT Primer Mix	50 µl	200 µl
lnR RT Enzyme Mix	25 µl	100 µl
10×lnR RT Buffer	50 µl	200 µl
RNase-Free ddH_2O	1 ml	2×1 ml

Storage

Store at -30~-15°C for up to one year.

Introduction

lnRcute lncRNA First-Strand cDNA Kit is specially developed for reverse transcription of long non-coding RNA (lncRNA). Compared with mRNA, lncRNA has the characteristics of lower abundance, larger difference in GC content, more complex secondary structure, etc., therefore it is difficult to achieve the desired reverse transcription results of lncRNA using traditional reverse transcription reagents. The lnRcute lncRNA First-Strand cDNA Kit contains gDNase which can efficiently remove the residual genomic DNA in RNA samples by incubating the RNA samples at 42°C for 3 minutes, and thus effectively avoid the interference of residual genomic DNA on the subsequent PCR detection results. The reverse transcriptase used in the lnR RT Enzyme Mix is FastKing RT Enzyme, which is a novel reverse transcriptase with a hydrophobic motif added by molecular modification, resulting in stronger RNA affinity and thermal stability, and further improves its reverse transcription efficiency and reaction rate. The synthesis of the first-strand cDNA can be completed in 15 minutes at 42°C, with at least 10 kb in length. In addition, the affinity between the novel enzyme and RNA is stronger, and in combination with the specially optimized buffer system and primer system, this kit is outstanding in the aspects of stress resistance and effective reverse transcription of RNA templates with high GC content, complex secondary structure and low abundance. This kit is especially suitable for the reverse transcription reaction of lncRNA with relatively low expression level and relatively complex secondary structure.

Product Highlights

Excellent performance: The reverse transcription efficiency can reach over 95%; The detection limit of total RNA can be as low as 10 ng, and the reverse transcription length can reach 10 kb.

Simple and fast: The reaction system can be simply set up, and the lncRNA first-strand cDNA can be synthesized within 21 minutes.

Wide applications: This kit can be widely used for RNA templates from various species, and for RNA templates with large GC content difference, complex secondary structure, low abundance and high level of impurities.

Good compatibility: Compatible with downstream applications such as RT-qPCR with high sensitivity and good stability.

Important Notes

1. The following protocol is applicable to the total RNA with the template amount of 10 ng-2 μg. If the total RNA amount is higher than 2 μg, please increase the reaction system volume in proportion.
2. Please operate on ice to prevent RNA degradation.
3. For RNA template with complex secondary structure, a denaturing step is recommended, that is, before starting, incubate the template RNA at 65°C for 5 minutes, then transfer to ice quickly to perform the next step.
4. According to different experimental needs, the Oligo-dT Primer or Gene Specific Primer can also be applied. The amount of primers is as follows: Oligo-dT Primer 50 pmol/ 20 μl reaction system, Gene Specific Primer 5 pmol/ 20 μl reaction system.
5. The non-specific amplification effect during the PCR reaction can be decreased by improving the reverse transcription reaction temperature to 50°C.

Protocol

Rapid synthesis of lncRNA first-strand cDNA by lnRcute lncRNA First-Strand cDNA Kit.

For 10 ng-2 μg total RNA, please set up a 20 μl reaction system. The procedure is as follows:

1. Thaw the template RNA on ice; Thaw 5×gDNA Buffer, lnR-RT Primer Mix, 10×lnR RT Buffer and RNase-Free ddH_2O at room temperature (15-25°C) and quickly place on ice. Vortex the solutions before use, and pulse-spin the tubes to collect the liquid on the tube wall.

   Note: Please perform the following steps on ice. To ensure the accuracy of the reaction mixture, it is recommended to prepare a mastermix first, then aliquot into each reaction tube.
2. Prepare the genomic DNA removal reaction mix according to table 1, and mix thoroughly by pipetting and pulse-spin the tube. Incubate at 42°C for 3 min, then quickly put on ice.

Table 1. gDNA removal reaction system

Component	Volume	Final Concentration
Total RNA	-	10 ng-2 μg
5×gDNA Buffer	2 μl	1×
RNase-Free ddH_2O	Up to 10 μl	-

3. Prepare the reverse transcription reaction mix according to table 2.

Table 2. Reverse transcription reaction system

Component	Volume	Final Concentration
10×lnR RT Buffer	2 μl	2×
lnR RT Enzyme Mix	1 μl	-
lnR-RT Primer Mix	2 μl	-
RNase-Free ddH_2O	Up to 10 μl	-

4. Transfer the reverse transcription reaction mix to the gDNA removal reaction mix from step 2, and thoroughly mix to make a 20 μl reaction system.
5. Incubate the reaction at 42°C for 15 min.
6. Incubate the reaction at 95°C for 3 min, then quickly transfer to ice. The cDNA product can be used for subsequent experiments, or stored at -20°C.

RNA templates requirements

Reverse transcriptase uses RNA as template to synthesize the first strand of cDNA. The quality and input amount of template RNA directly affect the results of reverse transcription.

1. Integrity of the template: The integrity of the template RNA is very important for reverse transcription. The remaining RNase in the RNA template will degrade the template RNA, resulting in low yield of cDNA products or even no cDNA products.
2. Purity of the template: Protein, salt ion, EDTA, ethanol, phenol or other impurities remaining in the RNA template will affect the activity of reverse transcriptase, thus affect the results of reverse transcription.
3. Input amount of the template: For 10 ng-2 μg of input template RNA, the above protocol is applicable. If the amount of template RNA is more than 2 μg, please increase the reaction system volume in proportion.

Important notes

1. If the follow-up experiment is RT-qPCR, the input amount of RT products should not exceed 1/10 of the final volume of PCR reaction system. For example, for 50 μl PCR reaction system, the input amount of RT products should not exceed 5 μl.
2. Upon the completion of reverse transcription, please place the cDNA products on ice before preparing the subsequent PCR reaction system. For long-term storage, please store at - 20°C.