Tinzyme Co., Limited

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1st strand cDNA transcription Kit, 100prep

Product Number: PCK5802

Shipping and Storage

Low temperature transportation, stored at -20℃, with a shelf life of one year.

Component

Component	100T
MMLV RTase, 200U/μL	100μL
RNase Inhibitor, 20U/μL	100μL
2×RT Buffer	1mL
RNase free water	1mL

Description

This kit provides all the reagents required for synthesizing the first strand of cDNA. The principle is to efficiently synthesize full-length cDNA copies of mRNA using mutated Moroni murine leukemia virus reverse transcriptase (M-MuLV RT).

Features

1. The use of mutated reverse transcriptase results in low endogenous RNase H activity.
2. Can synthesize cDNA with a length of 9kb or more.
3. Oligo dT and random primers can be used, which are provided in this kit.
4. Total RNA, poly (A)+RNA, or special RNA can all be used as templates.
5. Can be used for the synthesis of the second chain RT-PCR, Probe labeling, etc.

Note

1. The sample should avoid repeated freezing and thawing, otherwise it may lead to a decrease in the amount of nucleic acid extracted or a smaller number of extracted nucleic acid fragments.
2. Before use, check if there is any leakage of reagents in the pre installed plate. Before using the pre installed plate, please mix it upside down and immediately remove or gently shake it to avoid liquid accumulation.

Protocol

1. Recommended reaction conditions for the synthesis of the first strand of cDNA

1.1. Add 2×RT Buffer 10ull to a PCR tube without RNase; Total RNA (10ng-5μg) or mRNA (1-500ng) xμll; ddOH2 (9-x)μL.

1.2. After holding at 70℃ for 10 minutes, quickly take an ice bath for 2-10 minutes.

1.3. Centrifuge for a few seconds to allow the reaction solution to accumulate at the bottom of the PCR tube.

1.4. Add 1μL of enzyme mixture to the above reaction solution, gently pipette and mix well.

1.5. Keep at 50℃ for 30-50 minutes (mainly using Oligo dT12-18 or gene specific primers); If using random primers as the main method, keep at 25℃ for 10 minutes and at 45℃ for 50 minutes.

1.6. Incubate at 95℃ for 5 minutes and then cool on ice to obtain cDNA. This product can be directly used for the synthesis of the second chain or RT-PCR amplification reaction. Please store at -20℃.

Note: When the 2×RT buffer in the component is dissolved, white precipitation may occur, but it does not affect its quality. At this point, please use an oscillator or other equipment to mix it evenly, and wait until it is completely dissolved before use.