Gold Reverse Transcriptase

For research use only

Components

Component	R3001 (2,000 U)	R3002 (10,000 U)
5X Gold Buffer	100 µl	500 µl
Gold Reverse Transcriptase (200 U/µl)	10 µl	50 µl

Storage

This reagent should be kept at -30~-15°C.

Description

Gold Reverse Transcriptase is a new reverse transcriptase obtained by *in vitro* molecular evolution based on M-MLV (RNase H-) Reverse Transcriptase. The first strand of cDNA can be synthesized at 37~55°C. Gold Reverse Transcriptase has further significantly improved sensitivity, specificity, thermal stability and half-life, which is very suitable for reverse transcription of RNA templates with complex secondary structure. Gold Reverse Transcriptase has stronger polymerization and extension ability, which can be used for the synthesis of long cDNA and the construction of high proportion of full-length cDNA library.

Unit Definition

Poly (rA)·Oligo (dT) was used as the template/primer. At 37°C for 10 min, the amount of enzyme required to add 1 nmol dTTP as an acid-insoluble substance was defined as 1 unit of activity (U).

Matters Needing Attention

Prevent RNase contamination

Please keep the experimental area clean.Clean gloves and masks should be worn during operation. RNase-free shall be ensured for the centrifugal tubes, pipetting tips and other consumables used in the experiment.

Primers selection

The follow-up experiment is PCR

If the template is of eukaryotic origin, Oligo dT is generally preferred, paired with the 3 'Poly A tail of eukaryotic mRNA for maximum yield of full-length cDNA.

Gene specific primers (GSP) have the highest specificity.However, in some cases, the GSP used for PCR reaction cannot effectively guide the synthesis of the first strand of cDNA.At this point, the reverse transcription can be redone using Oligo dT or Random hexamers.

Random hexamers have the lowest specificity, and all RNA, including mRNA, rRNA, and tRNA, could be used as templates for Random hexamers.Random hexamers can be used as a primer when the target region has a complex secondary structure or a high GC content, or when the template is prokaryotic and the use of Oligo dT or gene-specific primers (GSP) cannot effectively guide cDNA synthesis.

The follow-up experiment is qPCR

Oligo dT mixed with Random hexamers resulted in the same cDNA synthesis efficiency in each region of the mRNA, helping to improve the authenticity and repeatability of the quantitative results.

Protocol

The synthesis of first-strand cDNA

1. Prepared the first cDNA synthesis reaction solution

Component	Amount	Final Conc.
Oligo (dT)15 Primer (50 µM) [1] or Random hexamers (100 µM) [1] or Gene Specific Primers (2 µM) [1]	1 µl	2.5 µM 5 µM 0.1 µM
Total RNA[2] or Poly A⁺ RNA[2]	10 pg ~ 1 µg 10 pg ~ 100 ng	0.5 pg ~ 50 ng/µl 0.5 pg ~ 5 ng/µl
5X Gold Buffer	4 µl	1X
dNTP Mix (10 mM each)	1 µl	0.5 mM
Gold Reverse Transcriptase (200 U/µl)	1 µl	10 U/µl
RNase inhibitor (40 U/µl)	1 µl	2 U/µl
RNase-free ddH_2O	to 20 µl	-

[1] Please select the appropriate primers according to your experimental needs: Oligo dT primer transcribes only mRNA, Random Primer transcribes all types of RNA, and specific primer transcribes only specific RNA fragments. When the subsequent experiment is qPCR, it is recommended to mix 1 µl each of Oligo dT and Random Primer to improve the accuracy of quantitative results.

[2] If the product is subjected to PCR reaction, it is recommended to denature the template RNA to eliminate the secondary structure first, which helps to improve the yield of the reverse transcription product. Prepare the following reaction system, and heated at 65°C for 5 min, then rapidly cooled on ice for 2 min:

Component	Amount
Total RNA or Poly A⁺ RNA	10 pg ~ 5 µg 10 pg ~ 500 ng
RNase-free ddH_2O	to 10 µl

2. Conduct the first-strand cDNA synthesis reaction according to the following conditions

Temperature	Products for PCR	Products for qPCR
	Time	
25°C [1]	5 min	/
37°C [2]	45 min	15 min
85°C	5 sec	5 sec

[1] This step is required only when using Random primer; This step is omitted when using Oligo dT Primer or Gene Specific Primer.

[2] If the template has a complex secondary structure or a high GC region, the reaction temperature

can be raised to 50°C, which can help increase the yield. The product can be used immediately for PCR/qPCR reaction or stored at -20°C and used within half a year. Repeated freeze-thaw of cDNA should be avoided.