CYPC1802-TRUEscript 1st Strand cDNA Synthesis Kit

(OneStep gDNA Removal)

Components	Size	
	CYPC1802 (50 rxns)	CYPC1803 (100 rxns)
5 × TRUE Reaction Mix	200 µl	400 µl
gDNA Remover	50 µl	100 µl
Oligo(dT) (0.5 µg /µl)	50 µl	100 µl
Random primer (N6)	50 µl	100 µl
RNase-free H_2O	1.5 ml	1.5 ml

Note: All reagents, when stored at -20 °C, are stable for 12 months.

Product Storage: Store at -20°C, stable for at least 12 months

Product Description

This product uses RNA as a template and employs a pre-mixing technique to efficiently synthesize the first-strand cDNA using the 5×TRUE Reaction Mix (pre-mixed with TRUEscript H^- RTase, RNase Inhibitor, dNTP Mixture, and Buffer). The operation is simple. This product utilizes a next-generation reverse transcriptase, generated by molecular evolution techniques with multiple site mutations, which exhibits enhanced extension ability and stability. It can be used for longer cDNA synthesis and construction of high-quality full-length cDNA libraries, and other kinds of related applications. The kit includes a special gDNA Remover with DNA degradation activity, allowing simultaneous genomic DNA removal and reverse transcription in a single step. This greatly simplifies the operation steps, avoids sample contamination and RNA degradation risks associated with complex sample addition processes.

Applications: First-strand cDNA synthesis. Suitable for cloning and detection of both high-copy and low-copy genes.

Product Features

1. The next-generation reverse transcriptase significantly improves stability and reverse transcription efficiency. It can synthesize cDNA with lengths up to 12 kb.

2. The fully pre-mixed reverse transcription Mix allows for simple and rapid completion of reverse transcription by only adding RNA, primers, and water.

3. The gDNA Remover employs advanced one-step genomic DNA removal technology, completing the removal of genomic DNA in the shortest time and simplest manner.

4. The RNA template volume can be up to 75% of the total reaction volume, making it suitable for reverse transcription reactions with low-concentration RNA templates.

5. 5 × TRUE Reaction Mix does not freeze at -20°C, reducing thawing and mixing time, making it easier to use.

6. Users can flexibly choose Oligo (dT), Random primer, or gene-specific primers as reverse transcription primers according to their needs.

Primer Selection

1. If the template is derived from eukaryotic organisms, Oligo (dT) is generally preferred as it pairs with the 3' PolyA tail of eukaryotic mRNA, yielding the highest yield of full-length cDNA.

2. If the presence of a polyA tail in mRNA cannot be confirmed for certain species, Oligo (dT) primer is the first choice. If unsuccessful, gene-specific primers (GSP) and Random primer can be attempted as primers.

3. Gene-specific primers (GSP) exhibit the highest specificity. However, in some cases, GSPs used for PCR may not effectively guide the synthesis of the first-strand cDNA. In such cases, Oligo (dT) or Random primer can be used for reverse transcription instead.

4. Random primers have the lowest specificity. Therefore, all RNA, including mRNA, rRNA, and tRNA can be used as templates for the Random primers. When the target region has complex secondary structures or high GC content, or the template is derived from prokaryotic organisms, Random primer can be used as a primer when Oligo (dT) or gene-specific primers (GSP) fail to effectively guide cDNA synthesis.

5. If the synthesized cDNA downstream is used for fluorescence quantitative PCR, mixing Oligo (dT) and Random primer (adding 1μl of each to a 20μl reaction system) can ensure equal efficiency of cDNA synthesis in different regions of mRNA, which helps improve the

authenticity and repeatability of quantitative results.

First-Strand cDNA Synthesis (using a 20μl reaction system as an example):

1. Add the following components (before use, gently tap or vortex each solution to mix briefly and centrifuge briefly to collect the liquid at the bottom of the tube):

Components	Volume
Total RNA/mRNA	50 ng-5μg/5-500 ng
Oligo(dT) (0.5μg/μl) or Random Primer (0.1μg/μl) or GSP (Gene Specific Primer, 2pmol/μl)	1μl
5 × TRUE Reaction Mix	4μl (see Note 4)
gDNA Remover *	1μl (see Note 4)
RNase-free H_2O	to 20μl (adjust to a total volume of 20 μl)

* Do not add gDNA Remover when the product is used for downstream gene cloning. If genomic DNA removal is not required during reverse transcription, omit the gDNA Remover component.

2. Gently mix.

If using Oligo(dT) or gene-specific primers (GSP), incubate at 42°C for 30-50 minutes (15 minutes if the product is used for qPCR).

If using Random Primer, incubate at 25°C for 10 minutes, then at 42°C for 30-50 minutes (15 minutes if the product is used for qPCR).

Note: If the template has complex secondary structures or regions with high GC content, try increasing the reaction temperature to 50°C to improve yield.

3. Heat at 85°C for 5 seconds to inactivate TRUEscript H^- RTase.

4. The obtained cDNA product can be used immediately for PCR or stored at -20°C for up to six months. For long-term storage, it is recommended to aliquot and store at -70°C. Avoid repeated freeze-thaw cycles for cDNA.

RT-PCR

It is recommended to use 1/10 to 1/5 volume (2-4 μl) of the reverse transcription product as a PCR template. For highly abundant samples, the cDNA can be appropriately diluted before use.

Recommended PCR Conditions

Please follow the instructions provided with the selected PCR reagents, such as those from Changyubio or other manufacturers.

Recommended Changyubio PCR Reagents

General amplification: CYPC09-2×Taq PCR Master Mix and CYPC80-2×F8 FastLong PCR Master Mix

High-fidelity amplification: CYPC82-2×A8 FastHiFi PCR Master Mix and CYPC84-2×A9 LongHiFi PCR Master Mix

Notes

1. Avoid RNase contamination.

2. To ensure successful reverse transcription, high-quality RNA samples are recommended.

3. Optional step (generally not necessary): If the RNA template has a high GC content, complex secondary structures, or the amplification of cDNA exceeds 3 kb, you can first mix only the RNA template, primers, and RNase-free H2O, denature at 65°C for 5 minutes, cool on ice, briefly centrifuge, and then add the remaining components to continue with the reverse transcription steps.

4. The 5 × TRUE Reaction Mix and gDNA Remover are viscous due to the presence of glycerol and tend to adhere to the tube walls and pipette tips, resulting in loss. Before use, spin down to avoid volume loss due to adhesion to the outer wall of the pipette tip. The enzymes in the 5 × TRUE Reaction Mix are in excess, so even if using 3.6μl-3.8μl each time and gDNA Remover at 0.8μl-0.9μl, the performance will not be affected.