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First Strand cDNA Synthesis Kit

Cat. No.: XV007PR-F (5 rxns), XV007PR (50 rxns)

Cat. No.: XV008PR (100 rxns), XV008PR-XL (250 rxns)

Introduction

XpressBio's First-Strand cDNA Synthesis Kit is a system that includes all the necessary components to synthesize first-strand cDNA, except the template RNA (total RNA or mRNA). The high-quality reverse transcriptase, ultrapure dNTPs and an optimized cDNA synthesis buffer ensure superior results with highest reproducibility. For greater application flexibility, hexamer primers, allowing all RNAs in the reaction to be used as templates, and an oligo (dT) primer, for the synthesis of cDNA from only poly(A) tailed mRNA, are included.

The synthesized single-stranded cDNA is suitable for real-time quantitative PCR applications. The kit has been formulated to provide high yields of full-length cDNA product and to increase sensitivity in RT-qPCR. Starting material can range from 10 pg to 1 µg of total RNA.

Application

- First strand cDNA synthesis for RT-PCR and RT-qPCR
- Construction of full-length cDNA libraries
- RNA analysis

Features

- Complete kit—all the components for the RT reaction are included.
- Full-length first strand cDNA up to 10kb.
- Formulated to increase sensitivity in RT-qPCR and RT-PCR assays.
- Reduced RNase H activity.
- Increased thermal stability from 37°C - 65°C.
- RNase inhibitor protects the RNA template from degradation.

Kit Contents

Components	XV007PR	XV008PR-XL
Reverse Transcriptase (200U/µl)	50 µl	5 x 50 µl
5X cDNA Synthesis Buffer	500 µl	5 x 500 µl
0.1 M DTT	250 µl	5 x 250 µl
dNTP Mix (10mM)	50 µl	5 x 50 µl
Hexamer primer (200 ng/µl)	50 µl	5 x 50 µl
Oligo $(dT)_{20}$ (50 µM)	50 µl	5 x 50 µl
RNase Inhibitor (50 U/µl)	25 µl	5 x 25 µl
Nuclease-free H_2O	1 ml	5 x 1 ml

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Storage

Upon receipt of the kit, immediately store the components at -20 °C in a freezer without a defrost cycle. It is recommended to reduce freeze-thaw cycles as less as possible.

Quality Control

The performance of the First-Strand cDNA Synthesis Kit is tested in an RT reaction using human total RNA. The sensitivity of the kit is verified by the detection of the GAPDH transcription in 100 fg total RNA and the product generated is visualized on agarose gel.

All preparations are assayed for contaminating endonucleases, exonucleases, nonspecific RNases, single and double-stranded DNase activities.

Protocol

1. Place 5X cDNA Synthesis Buffer at room temperature, thaw and vortex gently.
2. On ice, add the following reaction components into a sterile, nuclease-free tube:

RNA (10 ng – 5 µg)	X µl
Primers:	1 µl
Oligo $(dT)_{20}$ (50 µM) – or	
Hexamer primer (200 ng/µl) – or	
Gene-specific primer (10 µM)	
dNTP Mix (10 mM each)	1 µl
Nuclease-free H_2O	up to 12.5 µl

3. **Optional:** If GC-rich or structured RNA template is used, mix gently, centrifuge briefly and incubate 5 minutes at 65°C, then chill on ice.
4. Add the following components to the reaction tube in the indicated order:

5X cDNA Synthesis Buffer	4 µl
DTT 0.1M	2 µl
RNase Inhibitor (50 U/µl)	0.5 µl
Reverse Transcriptase (200 U/µl)	1 µl
Total Volume	20 µl

5. Transfer the sample to preheated, appropriate temperature thermal cycler. Incubate as follows:
   - Hexamer primer, incubate 10 minutes at 25°C followed by 50°C (or 37°C - 65°C) for 20–50 minutes.

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- Oligo (dT) or gene-specific primer, incubate at 50°C (or 37°C - 65°C) for 30–60 minutes.

  Note: *50°C is suitable temperature for most targets. For G-C rich RNA templates or with complex secondary structure, temperature can be increased to 65°C.*

6. Inactivate the reaction by heating at 85°C for 5 minutes, and then chill on ice.
7. The cDNA product should be stored at –20°C.