



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.
- Starting material: 10 pg – 1 µg of total RNA.
- Optimal reaction temperature: 55°C
- RNase inhibitor protects the RNA template from degradation.

Kit Contents

| Components | Size (50 rxns) |
|--------------------------------|----------------|
| Reverse Transcriptase | 50 µl |
| 5X cDNA Synthesis Buffer | 400 µl |
| dNTP Mix | 100 µl |
| Hexamer primer | 50 µl |
| Oligo (dT) primer | 25 µl |
| RNase Inhibitor | 25 µl |
| Nuclease-free H ₂ O | 2 x 1.25 ml |



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.

Protocol

1. On ice, add the following reaction components into a sterile, nuclease-free tube (for multiple reactions, a master mix without RNA may be prepared):

| | |
|--|------------------|
| dNTP Mix (10 mM each) | 2 μ L |
| RNase Inhibitor, 40 U/ μ l (optional) | 0.5 μ L |
| <i>Oligo (dT)12–18 (10 μM) – or</i> | 0.5 μ L |
| <i>Hexamer primer (25 μM) – or</i> | 1 μ L |
| <i>Gene-specific primer (10 μM)</i> | 0.5 μ L |
| 5x cDNA Synthesis Buffer | 4 μ L |
| RNA * | \leq 1 μ g |
| Reverse Transcriptase | 1 μ l |
| Nuclease-free H ₂ O | up to 20 μ L |

*0.1–1 μ g total RNA or 50–500 ng mRNA (polyA)

2. Mix and collect the drops by centrifuging briefly.
3. Incubation with:
 - Hexamer primer, incubate 10 minutes at 30°C followed by 50–55°C for 20–60 minutes
 - Oligo (dT) or gene-specific primer, incubate at 50–55°C for 20–60 minutes.
4. Inactivate the reaction by heating at 99 °C for 5 min, and then chill on ice.
5. The cDNA product should be stored at –20°C.

PRODUCT USE LIMITATION This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.