Data sheet

First Strand cDNA Synthesis Kit

Cat. No: PR007 (50 reactions)
Cat. No: PR008 (100 reactions)

Introduction

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). **First-Strand cDNA Synthesis Kit** features two optimized mixes, **RT Enzyme Mix** and **RT Reaction Mix**. RT Enzyme Mix combines Reverse Transcriptase and RNase Inhibitor, while RT Reaction Mix contains dNTPs, oligo$(dT)_{18}$ primers, random hexamers, $MgCl_2$ and an optimized buffer. 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 5 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 – 5 µg of total RNA.
- Optimal reaction temperature: 50°C
- RNase inhibitor protects the RNA template from degradation.
- Additional RNase H treatment may increase the sensitivity of the RT-qPCR reaction.

Kit Contents

Components	PR007	PR008
RT Enzyme Mix	100 µL	2X100µL
2X RT Reaction Mix	500 µL	2X500µL
RNase H (*E. coli*)	50 µL	100µL
Nuclease-free H_2O	1 mL	2X 1mL

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 **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 GAPDH transcript in 100 fg total RNA and the product generated is visualized on agarose gel.

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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):
   The reagents should be added in the following order:

2XRT Reaction Mix	10 µL
RT Enzyme Mix	2 µL
RNA	≤ 5 µg
Nuclease-free H_2O	up to 20 µL

2. Mix gently and incubate at 25 °C for 10 min.
3. Incubate at 50 °C for 30 min.
4. Inactivate the reaction by heating at 85 °C for 5 min, and then chill on ice.
   [Optional step] Add 1 µL of RNase H and incubate at 37 °C for 20 min.
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. Please refer to www.canvaxbiotech.com for Material Safety Data Sheet of the product.