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DATA SHEET

Version: 03
Revision date: 22/05/2023

1. Identification

Product name **First Strand cDNA Synthesis Kit**
100 rxn

Cat. No **PR008**

2. Description

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 is used for the preparation of cDNA libraries or for first strand cDNA synthesis for use in RT-PCR and RT-qPCR reactions.

3. Composition

Items	Quantity
Reverse Transcriptase (200U/µl)	2 x 50 µL
5X cDNA Synthesis Buffer	2 x 500 µL
0.1 M DTT	2 x 250 µL
dNTP Mix (10 mM)	2 x 50 µL
Hexamer primer(200ng/µl)	2 x 50 µL
Oligo (dT)20(50µM)	2 x 50 µL
RNase Inhibitor (50U/µl)	2 x 25 µL
Nuclease-free H2O	2 x 1 ml

4. 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.

5. Applications

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

6. 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 qPCR and PCR assays.
- ✓ Reduced RNase H activity.
- ✓ Increased thermal stability in the range of 37˚C to 65˚C.
- ✓ RNase inhibitor protects the RNA template from degradation.

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7. 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.
All preparations are assayed for contaminating endonucleases, exonucleases, nonspecific RNases, single and double-stranded DNase activities.

8. Protocol

1. Place 5 X 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-5µg)	X µL
Primers:	1 µL
Oligo (dT)20 (50 µM) – or	
Hexamer Primer (200ng/µl) – or	
Gene Specific Primer (10 µM)	
dNTP Mix (10 mM each)	1 µL
Nuclease-free H2O	up to 12.5 µL

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

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

5. Transfer the sample to preheated to appropriate temperature thermal cycler. Incubate as follows:
   - ✓ Hexamer Primer, incubate 10 minutes at 25˚C followed by 50˚C (or 37-65˚C) for 20–50 minutes.
   - ✓ Oligo (dT) or gene-specific Primer incubate at 50˚C (or 37-65˚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 min, and then chill on ice.
7. The cDNA product should be stored at –20˚C.

9. Further information

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

Disclaimer The information provided in this Data Sheet is correct to the best of our knowledge and belief at the date of publication. This information is intended only as a guide and should not be taken as a warranty or quality specification. Canvax Reagents S.L.U. shall not be held liable for any damage resulting from handling or from contact with the above product.