NZY First-Strand cDNA Synthesis Kit

Catalogue number: MB12501, 50 reactions
MB12502, 250 reactions

Features

- Provides high yields of full-length cDNA products for use in RT-qPCR and two-step RT-PCR assays
- Formulated to increase sensitivity in RT-qPCR
- Primer type: oligo(dT)$_{18}$ and random hexamers
- Starting material: 10 pg to 5 µg of total RNA
- Optimal reaction temperature: 50 °C
- Convenient and reliable

Description

NZY First-Strand cDNA Synthesis Kit is a system that includes all the necessary components to synthesize first-strand cDNA, except the template RNA.

The resulting single-stranded cDNA is suitable for use in real-time quantitative Reverse Transcription PCR (RT-qPCR). NZY First-Strand cDNA Synthesis Kit is formulated to provide high yields of full-length cDNA products and to increase sensitivity in RT-qPCR.

Starting material can range from 10 pg up to 5 µg of total RNA. The kit includes a combination of random hexamers and oligo(dT)$_{18}$ primers in order to increase sensitivity. The primers are included in the NZYRT 2× Master Mix, which also contains dNTPs, $MgCl_2$ and an optimized RT buffer. NZYRT Enzyme Mix includes both NZY Reverse Transcriptase (RNase H minus) and NZY Ribonuclease Inhibitor in order to protect RNA against degradation due to ribonuclease contamination. RNase H (from *E. coli*) is provided in a separate tube to specifically degrade the RNA template in cDNA:RNA hybrids after the first-strand cDNA synthesis. This procedure will improve the sensitivity of subsequent RT-qPCR reaction since PCR primers will bind more easily to the cDNA.

Shipping conditions

NZY First-Strand cDNA Synthesis Kit is shipped on dry ice.

Storage conditions

Store all kit components at -20 °C in a freezer without defrost cycles. Stability can be extended by storing it at -80 °C. The kit is stable for up to 3 years.

System Components

Component	MB12501 (50 reactions)	MB12502 (250 reactions)
NZYRT Enzyme Mix (1)	100 µL	5 × 100 µL
NZYRT 2× Master Mix (2)	500 µL	5 × 500 µL
NZY RNase H (*E. coli*)	50 µL	5 × 50 µL
DEPC-treated H_2O	1 mL	2 × 1 mL

(1) Includes NZY Reverse Transcriptase and NZY Ribonuclease Inhibitor
(2) Includes oligo(dT)$_{18}$, random hexamers, $MgCl_2$ and dNTPs

Protocol for first-strand cDNA synthesis

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

NZYRT 2× Master Mix	10 µL
NZYRT Enzyme Mix	2 µL
RNA (up to 5 µg)	× µL
DEPC-treated 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.

5. Add 1 µL of NZY RNase H (*E. coli*) and incubate at 37 °C for 20 min.

6. Use the cDNA product directly in PCR or qPCR diluted in TE buffer or undiluted; or store at -20 °C until required.

Important notes

- High quality intact RNA, free of residual genomic DNA and RNases is essential for full-length, high quality cDNA synthesis and accurate RNA quantification. For this reason, special precautions should be taken when working with RNA:
  - Aseptic conditions should be maintained: always wear gloves; change gloves whenever you suspect

that they are contaminated; use RNase-free tubes and pipet tips; designate a special area and equipment for RNA work only.
    - DNase I (not provided) may be used to eliminate genomic DNA contamination from the starting total RNA.
    - The template RNA should be stored at -70 °C. Avoid multiple freeze/thaw cycles of RNA.
- This kit does not include control RNA.
- Keep all reagents of the kit on ice while setting up the reactions.
- When performing RT-qPCR using the synthesized cDNA as template, no more than 1/10 of the final PCR volume should be derived from the reverse-transcription product. For instance, use up to 5 µL of cDNA obtained in the first-strand synthesis in a 50 µL PCR reaction.

Quality control assays

Purity

NZY Reverse Transcriptase and NZY Ribonuclease Inhibitor present in the Enzyme Mix are >90% pure as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie blue staining.

Nuclease assays

All components of the kit are tested for DNase and RNase activities, using 0.2-0.3 µg of pNZY28 plasmid DNA and 1 µg of RNA, respectively. Following incubation at 37 °C, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids.

Functional assay

NZY First-Strand cDNA Synthesis Kit is tested functionally in a RT-PCR experiment designed to calculate the number of mRNA copies of the GAPDH gene in mouse liver cells. Precisely, 0.5 µg of total RNA extracted from mouse liver is used as starting template material.

Troubleshooting

Little or no RT-PCR/RT-qPCR amplification product
• RNA damage or degradation
Analyse RNA on a denaturing gel to verify integrity. Use aseptic conditions while working with RNA to prevent RNase contamination. Replace RNA if necessary.
• Presence of RT inhibitors
Some inhibitors of RT enzymes include: SDS, EDTA, glycerol, sodium phosphate, spermidine, formamide and guanidine salts. Remove inhibitors by ethanol precipitation of the RNA preparation before use; wash the pellet with 70% (v/v) ethanol.
• Not enough starting RNA
Increase the concentration of starting RNA.
Unexpected bands after electrophoretic analysis of amplified products
• Genomic DNA contamination
DNase I may be used to eliminate genomic DNA contamination from the starting RNA.

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Certificate of Analysis

Test	Result
Enzyme purity	Pass
Nucleases assay	Pass
Functional assay	Pass

Approved by: José Prates

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