NZY First-Strand cDNA Synthesis Kit, separate oligos

Catalogue number:

MB17001, 50 reactions
MB17002, 250 reactions

Features

- Possibility to choose the primer to initiate the reaction
- 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
- Starting material: 1 ng to 5 µg of total RNA
- Optimal reaction temperature: 50 °C
- Convenient and reliable

Description

NZY First-Strand cDNA Synthesis Kit, separate oligos, is a system that includes all the necessary components to synthesize first-strand cDNA, except the template RNA. Random hexamers and Oligo(dT)$_{18}$ primers are provided in separate tubes to offer the convenience to choose the appropriate primer to initiate your reverse-transcription reaction.

The resulting single-stranded cDNA is suitable for use in real-time quantitative Reverse Transcription PCR (RT-qPCR). NZY First-Strand cDNA Synthesis Kit, separate oligos, 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. Besides random hexamers and Oligo(dT)$_{18}$ primers, the kit includes NZYRT 2× Master Mix, no oligos, which contains dNTPs, $MgCl_2$ and an optimized RT buffer; 10× Annealing Buffer and NZYRT Enzyme Mix. 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 reactions since PCR primers will bind more easily to the cDNA.

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 will remain stable till the expiry date if stored as specified.

System Components

Component	MB17001 (50 reactions)	MB17002 (250 reactions)
NZYRT Enzyme Mix (1)	100 µL	5 × 100 µL
NZYRT 2× Master Mix, no oligos (2)	500 µL	5 × 500 µL
10× Annealing Buffer	50 µL	5 × 50 µL
Random hexamer mix (50 ng/µL)	500 µL	500 µL
Oligo(dT)$_{18}$ primer mix (50 µM)	100 µL	3 × 100 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 $MgCl_2$ and dNTPs.

Protocol for first-strand cDNA synthesis

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

RNA (up to 5 µg)	× µL
Oligo(dT)$_{18}$ primer mix (50 µM), or random hexamer mix (50 ng/µL), or gene-specific primer (2 µM)	1 µL
10× Annealing Buffer	1 µL
Nuclease-free water	up to 8 µL

2. Mix gently and incubate at 65 °C for 5 min.

3. Place on ice for at least 1 min and then centrifuge briefly.

4. On ice, perform the reverse-transcription step, by adding the following components to the tube:

NZYRT 2× Master Mix, no oligos	10 µL
NZYRT Enzyme Mix	2 µL
Final Volume	20 µL

5. Mix gently and centrifuge briefly.

6. Incubate at 50 °C for 30 min.
Note: *When using random-hexamer primers, incubate first at 25 °C for 10 min and then proceed with the 30 min reaction step (step 6).*

7. Inactivate the reaction by heating at 85 °C for 5 min and then chill on ice.

8. Add 1 µL of NZY RNase H and incubate at 37 °C for 20 min.
Note: *Addition of NZY RNase H will remove RNA bond to cDNA. This procedure is mainly recommended when using cDNA in PCR amplification, especially for some targets (> 1 kb) that may require RNA-free DNA as template. RNA removal will increase the sensitivity of the PCR step.*

9. 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. Thus, special precautions should be taken:
  - 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 BlueSafe staining.

Nucleases assays

All components of the kit are tested for DNase and RNase contamination, 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 separate oligos is tested for performance in a RT-qPCR experiment using a 10-fold serial dilution of total RNA from mouse liver (1 µg to 0.1 ng). The resultant cDNA is then used as template in a quantitative real-time PCR assay using specific primers to amplify the mouse GAPDH gene.

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.

V2001

Certificate of Analysis

Test	Result
Enzyme purity	Pass
Nucleases assays	Pass
Functional assay	Pass

Approved by:

Patrícia Ponte
Senior Manager, Quality Systems

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