NZY First-Strand cDNA Synthesis Flexible Pack

Catalogue number:

MB40001, 200 reactions

Description

NZY First-Strand cDNA Synthesis Flexible Pack provides robust transcription of RNA as it includes all the necessary components to synthesize first-strand cDNA from an RNA template. This optimized pack includes the following components: our modified recombinant form of the Moloney Murine Leukemia Virus (M-MuLV) Reverse Transcriptase (RNase H minus) that synthesizes the complementary DNA strand using either RNA or single-stranded DNA as template in a wide range of temperatures (37-50°C); an optimized Reverse Transcriptases buffer; a NZY Ribonuclease Inhibitor for RNA protection against degradation due to ribonuclease contamination; a dNTPs mix; random hexamers and oligo(dT)$_{18}$ primers; and finally, an *E. coli* RNase H to specifically degrade the RNA template in cDNA-RNA hybrids after the first-strand cDNA synthesis. This flexible pack offers the possibility to choose the primer to initiate the reaction: you can choose either the primers provided in separate tubes, or a gene-specific primer. The pack is sufficient for up to 200 first-strand cDNA synthesis reactions and it is optimized for RT reactions over a wide range of total RNA concentrations from 10 pg to 5 µg. Downstream applications include real-time PCR, standard PCR and microarrays.

Storage conditions

Store all pack components at -20 °C in a freezer without defrost cycles. Stability can be extended by storing it at -80 °C. The pack will remain stable till the expiry date if stored as specified.

Pack Components

Component	MB40001 (200 reactions)
NZY Reverse Transcriptase (200 U/µL)	2 tubes
10x Reaction buffer for Reverse Transcriptases[1]	1 tube
NZY Ribonuclease Inhibitor (40 U/µL)	1 tube
dNTP Mix (10 mM each)	1 tube
Random hexamer mix (50 ng/µL)	1 tube
Oligo(dT)$_{18}$ primer mix (50 µM)	2 tubes
NZY RNase H (*E. coli*) (5 U/µL)	1 tube
DEPC-treated H_2O	3 tubes

[1] *Upon thawing, if any precipitate is observed, pulse vortex until the precipitate is completely resuspended.*

Protocol for first-strand cDNA synthesis

On ice, in a sterile, nuclease-free microcentrifuge tube, prepare a reaction mixture, combining the following components:

total RNA or mRNA / poly(A) RNA	10 pg – 5 µg 10 pg – 500 ng
Oligo(dT)$_{12-18}$, and/or Random hexamer mix, or gene-specific primer	x µL (50 µM) x µL (50 – 250 ng) x µL (2 pmol)
dNTP Mix (10 mM each)	x µL (0.5 mM final concentration)
DEPC-treated H_2O	up to 16 µL

Note: *For two-step RT-PCR experiments, a mixture of oligo(dT) and random primers is highly recommended to achieve the benefits of each primer type, and thus to increase the sensitivity of cDNA synthesis reaction.*

2. For some GC-rich RNAs or nucleic acids with high degree of secondary structure, a denaturation step may be required. If so, centrifuge briefly and incubate the mix at 65 °C for 5 min. Chill on ice for at least 1 min, briefly centrifuge again and place on ice.

3. Add the following reaction components:

10× Reaction Buffer	2 µL
NZY Ribonuclease Inhibitor	1 µL
NZY Reverse Transcriptase	1 µL (200 units)
Final Volume	20 µL

4. Mix gently and centrifuge briefly.

5. Incubate at 50 °C for 30-50 min.

Note: *When using random-hexamer primers, incubate first at 25 °C for 10 min and then proceed with the 30-50 min reaction step (step 5).*

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

7. 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.*

8. Store cDNA product at -20 °C or proceed to downstream applications diluted in TE buffer or undiluted.

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 -80 °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.
- The resulting cDNA can be stored at -20 °C or at 4 °C for up to one week. If long-term storage is required, -80 °C is recommended. Avoid freeze/thaw cycles of the cDNA.

- cDNA can be used for cloning or as a template in PCR or qPCR reactions. Typically, 10% (2 µL) of the first-strand reaction is enough for most PCR applications. Optionally, the cDNA can be diluted in TE buffer.

Quality control assays

Purity

NZY Reverse Transcriptase, NZY Ribonuclease Inhibitor and RNase H are >90% pure as judged by SDS polyacrylamide gel electrophoresis followed by BlueSafe staining.

Nucleases assays

All components of the pack are tested for DNase and RNase contaminations, 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. NZY RNase H is tested only for DNase contamination.

Functional assay

NZY First-Strand cDNA Synthesis Flexible Pack is tested functionally in a RT-PCR 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.

Related products

Product name	Cat. No.
Agarose (routine grade)	MB144
GreenSafe Premium	MB132
NZYSpeedy qPCR Green Master Mix (2x), no ROX/ ROX / ROX plus	MB224 / MB223 / MB222
NZYSpeedy qPCR Probe Master Mix (2x), no ROX/ ROX / ROX plus	MB230 / MB229 / MB228

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. Ensure the use of NZY Ribonuclease Inhibitor; the addition of this inhibitor is essential when using less than 50 ng of RNA in order to safeguard the template against degradation due to ribonuclease 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. They can be problematic in smaller reaction volumes. If necessary, 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 (pre-treatment RNA). The enzyme volume should not exceed 10% of the total reaction volume.

V2001

Certificate of Analysis

Test	Result
Enzyme purity	Pass
Nucleases assays	Pass
Functional assay	Pass

Approved by:

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