MB129_IFU_EN_V2401

Random hexamer mix

Catalogue number	Presentation
MB12901	25 µg (500 µL)

Description

Random hexamer mix includes oligonucleotides representing all possible hexamer sequences. Random hexamers are commonly used to prime single-stranded DNA or RNA for extension by DNA polymerase or reverse transcriptase, particularly when the template is difficult to copy in its entirety or for RNA lacking a polyA tail, such as bacterial RNA. During cDNA synthesis, random hexamer will random priming throughout the entire length of the RNA to generate a cDNA pool containing various lengths of cDNA. With this method, all RNAs present in a population constitute templates for the cDNA synthesis experiment.

Applications of Random hexamer mix include cDNA synthesis using a Reverse Transcriptase (RT reaction), DNA synthesis using Klenow Fragment of DNA polymerase I or DNA probe synthesis.

Shipping & Storage Conditions

This product is shipped in dry ice. Upon receipt, store at -85 °C to -15 °C in a constant temperature freezer. Immediately after use, swiftly return it to a temperature between -85 °C and -15 °C to reduce exposure to room temperature. Minimize the number of freeze-thaw cycles by aliquoting smaller volumes after first thawing. Adhering to these meticulous storage procedures ensures that the random hexamer mix will remain stable until the expiry date and deliver reliable and consistent performance in all applications

Components

	MB12901	
COMPONENT	**TUBES**	**VOLUME**
Random hexamer mix	1	500 µL

Specifications

Concentration: 50 ng/µL (25.25 µM).

Primer sequence: 5'- d (NNNNNN)-3', where N = G, A, T or C

Standard Protocol

Procedure for first-strand cDNA synthesis

1. Thaw the Random hexamer mix on ice.
2. Mix gently, but thoroughly, to ensure that the solution is homogeneous.
3. Briefly centrifuge to spin down the contents
4. Use 1-5 µL in a 20 µL-reverse transcription reaction (50-250 ng/reaction).

Note: *The sensitivity of cDNA synthesis may be improved when using a mixture of random hexamer and oligo(dT)$_{18}$ primers (NZYtech, Cat. No. MB12801).*

Technical Notes

Priming methods for reverse transcription

Random hexamers are preferred for long transcripts or if they contain significant secondary structures. In addition, random hexamers are used for non-polyadenylated target templates (as prokaryotic mRNA). They will perform random priming throughout the entire length of the RNA to generate a cDNA pool containing various lengths of cDNA. With this method, all RNAs present in a population constitute templates for cDNA synthesis experiment. NZYtech provides a Random hexamer mix that includes oligonucleotides representing all possible hexamer sequences.

In addition to Random hexamer primers, alternative priming methods are available for reverse transcription. Below is an overview of these methods:

Oligo(dT) primers: specifically bind to the poly(A)-tail found at the 3´-end of most eukaryotic mRNAs. The capacity to generate many different cDNAs from the same starting RNA pool, makes these primers the preferred choice for two-step RT-PCR reactions. Different types of oligo(dT)s are available. Oligo $(dT)_{18}$ primer mix, a homogenous mixture of 18-mer thymidines, is available at NZYtech for the synthesis of full-length cDNA from poly(A)-tailed mRNA. In contrast to the standard oligo (dT), which randomly bind within the poly(A) tail of the eukaryotic mRNA, the anchored oligo(dT) primers bind at the beginning of the tail. This avoids unnecessary reverse transcription of this often-lengthy region, as well as the synthesis of erroneous products due to mispriming.

Gene-specific primers (GSPs): enhance sensitivity by allowing the reverse transcription of a specific RNA sequence. This priming method is chosen to perform one-step RT-PCR reactions once the same primer is used in both the RT and PCR steps. However, GSPs offer less flexibility than oligo(dT) and random primers since each cDNA synthesis is limited to one target gene.

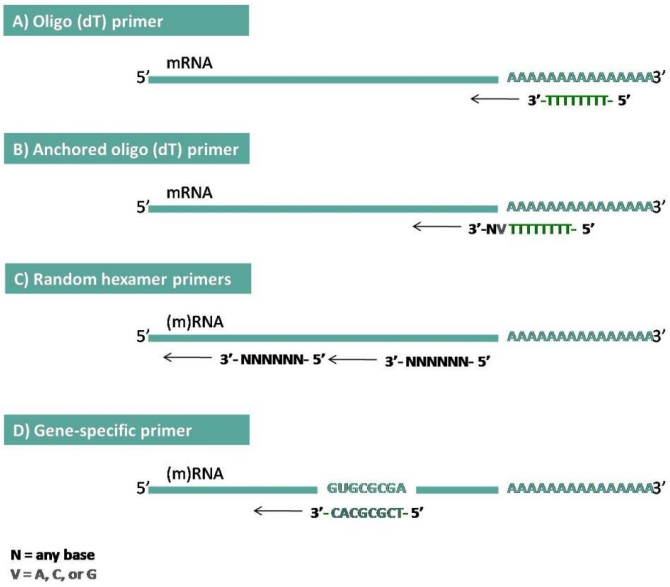

Figure 1. Schematic representation of priming methods for reverse transcription.

Quality Controls

Nuclease assays

To test for DNase contamination, 0.2-0.3 µg of pNZY28 plasmid DNA are incubated with 1 µL of Random hexamer mix for 14-16 h at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 1 µL of Random hexamer mix for 1 h at 37 °C. Following incubation, 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

Random hexamer mix is tested for performance in a RT-qPCR experiment using total RNA from mouse liver. After the first-strand cDNA synthesis with NZY Reverse Transcriptase, the resultant cDNA is then used as template in a quantitative real-time PCR assay using specific primers to amplify a housekeeping gene.