

Oligo (dT)₁₈ primer mix

Catalogue number	Presentation
MB12801	27 µg (100 µL)

Description

Oligo (dT)₁₈ primer mix is a synthetic single-stranded 18-mer oligonucleotide with 5'- and 3'-hydroxyl ends. Oligo (dT)₁₈ primer is suitable for use as a primer for first-strand cDNA synthesis with a reverse transcriptase. The primer hybridizes to the poly(A) tail found at the 3'-end of most eukaryotic mRNAs, making it more specific than random hexamers, as it will not transcribe rRNA. Oligo (dT)₁₈ primer is frequently employed when cDNA is used for cloning, cDNA library construction and qPCR.

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

COMPONENT	MB12801	
	TUBES	VOLUME
Oligo (dT) ₁₈ primer mix	1	100 µL

Specifications

Concentration: 270 ng/µL (50 µM).

Primer sequence: 5'- d (TTT TTT TTT TTT TTT TTT)-3'

Standard Protocol

Procedure for first-strand cDNA synthesis

1. Thaw the Oligo (dT)₁₈ primer 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 µL in a 20 µL reverse transcription reaction.

Note: The sensitivity of cDNA synthesis may be improved when using a mixture of oligo(dT)₁₈ primer and random hexamers (Nzytech, Cat. No. MB12901).

Technical Notes

Priming methods for reverse transcription

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.

In addition to oligo(dT) primers, alternative priming methods are available for reverse transcription. Below is an overview of these methods:

Random hexamers: 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 (Cat. No. MB129) that includes oligonucleotides representing all possible hexamer sequences.

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.

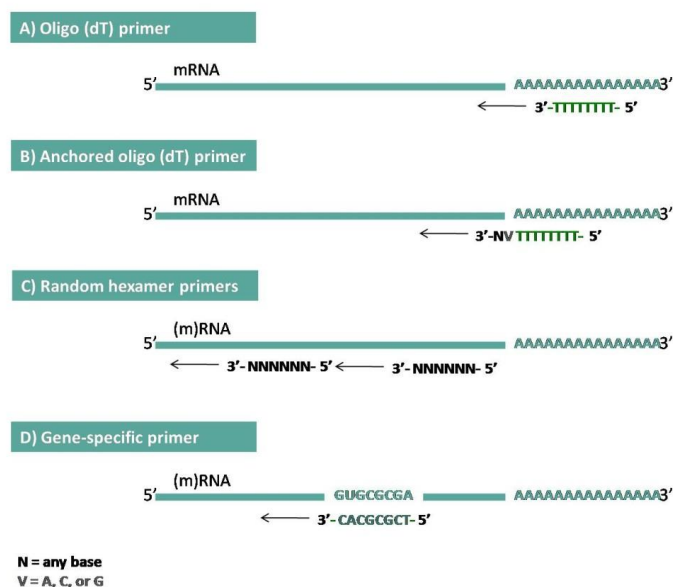


Figure 1. Schematic representation of priming methods for reverse transcription.

Quality Controls

Nuclease assays

To test for DNase contamination, 0.2-0.3 µg of pNZY28 plasmid DNA are incubated with 1 µL of Oligo (dT)₁₈ primer for 14-16 h at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 1 µL of Oligo (dT)₁₈ primer 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

Oligo (dT)₁₈ primer 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.

For life science research only. Not for use in diagnostic procedures.