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Oligo $(dT)_{20}$ Primer

Product	Cat#	Package size
Oligo $(dT)_{20}$ Primer (163.5µg / 27.2nmol)	**M3039.0155**	**5 OD**
Oligo $(dT)_{20}$ Primer (32.7µg / 5.4nmol)	**M3039.0151**	**1 OD**

Product description

Oligo $(dT)_{20}$ are single stranded oligodeoxyribonucleotides containing only deoxythymine (dT) to be able to prime with the poly(A) tail of mRNA molecules. Primers are quality controlled (MS), purified (reversed phase HPLC), aliquoted and lyophilized and came with a guaranteed amount of 1OD (about 5.4nmol (32.7µg)) or 5OD (about 27nmol (163.5µg)).

Oligo $(dT)_{20}$ Primer is designed to initiate the synthesis of cDNA from total RNA in a reverse transcription reaction, where Reverse Transcriptase is starting the reaction from the poly-A-end of mRNA. It can also be used for generation of labeled cDNA to screen microarrays.

A mixture (1:1 ratio (w/w)) of random hexamer primers and Oligo$(dT)_{20}$ primer may improve the sensitivity of cDNA synthesis as it will especially improve the efficiency of transcripts longer than 600 bp.

Applications

Oligo $(dT)_{20}$ Primer is suitable for use as a primer for first strand cDNA synthesis with a reverse transcriptase, such as MMuLV or AMV. The primer hybridizes to the poly-adenylated tail found on the 3´ end of most eukaryotic mRNAs. Oligo $(dT)_{20}$ ensures that the 3´ end of mRNAs are represented.

Oligo$(dT)_{20}$ Primer may be better suited for the new generation of Reverse Transcriptases like SuperScript™ that work at higher temperatures as the longer Oligo$(dT)_{20}$ Primer enable annealing in reverse transcription reactions at higher temperatures.

- cDNA synthesis from total RNA in a reverse transcription reaction.
- optimal choice for construction of cDNA libraries from eukaryotic mRNAs.
- Full length cDNA cloning
- 3' rapid amplification of cDNA ends (3' RACE)
- Generation of labelled cDNA for screening of microarrays.

Oligo $(dT)_{20}$ Primer cannot be used together with degraded RNA, prokaryotic RNA or miRNA (lack of poly(A) tail).

Primer Sequence: 5´ – d (TTT TTT TTT TTT TTT TTT TT) –3´

Amount / Concentration: lyophilized with an amount of 5 OD (ca. = 27.2nmol = 163.5µg).

Purity: HPLC purified and MS checked.

Preparation of working solution: for 5OD tube
Dissolve content (5 OD) in 271.5µL of molecular biology grade water for an end concentration of 100pmol/µL (100µM) with 100pmol/µL equal about 600ng/µL.
272µL (100µM / 600ng/µL) are sufficient for 325 up to 645 x 20µL RT reactions (250ng to 500ng Oligo $(dT)_{20}$ per 1µg RNA in a 20µL reaction volume).

Preparation of working solution: for 1OD tube
Dissolve content (1 OD) in 54.4µL of molecular biology grade water for an end concentration of 100pmol/µL (100µM) with 100pmol/µL equal about 600ng/µL.
54.4µL (100µM / 600ng/µL) are sufficient for 65 up to 129 x 20µL RT reactions (250ng to 500ng Oligo $(dT)_{20}$ per 1µg RNA in a 20µL reaction volume).

Stability and Storage: Oligo $(dT)_{20}$ Primers are stable for more than 24 months if stored at +2°C to +8°C.
Shipped at RT, store after delivery at +2°C to +8°C.

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Exemplary RT reaction protocol

1: Mix in the tube:

- 1 – 5µg of the total RNA (or 50 – 500ng of poly(A)-RNA)
- 10 pmol of strand-specific primer (or 250ng – 500ng of oligo-dT for each µg of RNA)
- add water up to 8µL

2: Incubate the mixture 10 min at 70°C, then 10 – 15 min at room temperature (for the specific primer) or place on ice in case of oligo-dT or random primer.

3: Add into the mixture:

- 4µL of 5x RT buffer complete
- 1µL of dNTP mix (10mM of each dNTP)
- RNase Inhibitor: 20 - 40 units (optional)
- 200 units MMuLV reverse transcriptase
- Fill in H2O to volume 20µL

4: Incubate the mixture at 37°C – 55°C for 30 – 120 min.
The time of reaction depends on the cDNA length, 30 min for cDNA in range of 500 bp length, 120 min for cDNA more than 1.5 kb.
The temperature of the reaction depends on the structural features of RNA. Use increased temperature (up to 55°C) for the highly structured RNA.
The optimal temperature and reaction time should be adjusted for each particular RNA.

5: Heat the mixture 10 min at 65°C – 70°C to inactivate the MMuLV.

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