GENAXXON bioscience

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Random Hexamer Primer N6

random 5'-hydroxyl hexanucleotides

Product	Cat#	Package size
Random Hexamer Primer N6 (136µg / 76nmol)	**M3038.0125**	**5 OD**
Random Hexamer Primer N6 (27.27µg / 15.2nmol)	**M3038.0121**	**1 OD**

Product description

Random Hexamers are short oligodeoxyribonucleotides of random sequence [d(N)6]. Primers are quality controlled (MS), purified (reversed phase HPLC), lyophilized and aliquoted and came with a guaranteed amount of at least 5OD (about 76.1nmol, respective 136.4µg)).

Hexanucleotide primers are a mixture of random 5'-hydroxyl hexanucleotides or hexamers and can be used to prepare radioactive or non-radioactive probes using a DNA polymerase and a suitable DNA template quickly and efficiently or for cDNA synthesis from mRNA. The heterogeneous nature of the random primers ensures that all possible sequences will be represented in the probe mixture.

An increase of the amount of random hexamers in the RT mixture may improve the amount of synthesized cDNA.

Applications

Random Hexamer Primer N6 are short oligodeoxyribonucleotides of random sequence [d$(N)_6$] that anneal to random complementary sites on a target DNA or RNA, to serve as primers for DNA synthesis by a DNA polymerase or reverse transcriptase.

- DNA synthesis using Klenow fragment with DNA templates
- DNA probe synthesis for use in Northern and Southern blots, and *in situ* hybridization applications
- Partially degraded RNA samples
- RNA without poly(A) tail such as ribosomal RNAs
- RNA with strong secondary structure
- Target regions at 5´ end of a long messenger RNA transcript

Primer Sequence:	5´ – d (NNNNNN) –3´ N = G, A, T or C
Amount / Concentration:	lyophilized powder with an amount of 5 OD (ca. = 76.1nmol = 136.4µg).
Preparation of working solution:	Dissolve content (5 OD) in 760µL of molecular biology grade water for an end concentration of 100pmol/µL (100µM) with 100pmol/µL equal 0.180µg/µL respective 180ng/µL If the random hexamers are used between 50ng and 250ng per 20µL RT reaction the content of one tube supplied (136.4µg) will last for 460 up to 2300 reverse transcription reactions.
Purity:	HPLC purified, and MS checked.
Stability and Storage:	Random Hexamer Primers N6 are stable for more than 24 months if stored at +2°C to +8°C. Shipment: not cooled. Stored 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 50ng – 250ng of random Hexamers for each µg of RNA)
- add water up to 8µL

2: Denature the RNA by incubating the mixture 10 min at 70°C. Then let it rest at 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 RNA.

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

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