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ProductInformation

Oligo(dT)$_{23}$ Primers, Anchored

Product No. **O 4387**
Store at –20 °C

Product Description

Sigma's anchored oligo(dT)$_{23}$ primers are used to prime mRNA with a poly(A) tail for cDNA synthesis. The primers have 23 thymidine residues and one G, C or A residue (the anchor) at the 3' end. This anchor insures that the oligo(dT) primer binds at the very beginning of the message and there is not a long region of unusable sequence. The anchored oligo (dT)$_{23}$ can be used in conjunction with random nonamers (Product No. R 7647) when preparing cDNA libraries, when sequence information is incomplete or absent, or other instances where specific primers are not useful. Random nonamers and anchored oligo(dT)$_{23}$ primers are provided as alternatives to specific RT primers for first strand synthesis, cDNA library construction and other applications. The anchored oligo(dT)$_{23}$ primers will not affect PCR† after transcription due to their decreased ability to prime at increased temperatures (up to 65 °C). Sigma provides anchored oligo(dT)$_{23}$ primers as a 0.5 µg/µl (70 µM) solution in water. A final concentration range of 1 µM to 3.5 µM for the anchored oligo(dT)$_{23}$ primers is recommended in the enhanced AMV reverse transcriptase procedure that follows.

Precautions and Disclaimer

For laboratory use only. Not for drug, household or other uses.

Reagents Required but Not Provided
(Sigma product numbers are given where appropriate)

- Deoxynucleotide Mix, Product No. D 7295, 10 mM dATP, 10 mM dCTP, 10 mM dGTP, 10 mM TTP
- RNase Inhibitor, Product No. R 2520
- Enhanced AMV Reverse Transcriptase, Product No. A 4464
- 10X Buffer for AMV Reverse Transcriptase, Product No. B 1175 (included with Enhanced AMV Reverse Transcriptase)
- Water, PCR Reagent, Product No. W 1754
- RNA to be transcribed and amplified
- Dedicated pipets
- Aerosol resistant pipet tips
- 0.5 ml or 0.2 ml thin-walled PCR tubes
- Thermal cycler

Procedure

The optimal conditions for the concentration of enhanced AMV reverse transcriptase, template RNA, primers and amplification parameters will depend on the system being utilized and should be determined empirically.

1. Add the following reagents to a thin-walled 200 or 500 µl PCR microcentrifuge tube on ice:

Volume	Reagent	Final Concentration
X µl	RNA template	In general, use 0.005-0.25 µg/µl total RNA or desired amount of poly(A)$^+$ RNA
1 µl	Oligo(dT)$_{23}$ primers, anchored	3.5 µM (Recommended final concen-tration range is 1 µM and 3.5 µM)
q.s.	Water	-------
10 µl	Total Volume	

2. Mix gently and briefly centrifuge to collect all components to the bottom of the tube.

3. Place tube in thermal cycler at 70 °C for 10 minutes.

4. Remove tube, place on ice, centrifuge and add the following components to the reaction:

Volume	Reagent	Final Concentration
2 µl	10X buffer for eAMV-RT	1X
1 µl	Deoxynucleotide mix	500 µM each dNTP
1 µl	Enhanced AMV reverse transcriptase	1 U/µl
1 µl	RNase inhibitor, diluted to 20 U/µl	1 U/µl
5 µl	Water	----
20 µl	Total Volume	

5. (Optional) Incubate the reaction tubes at 25 °C for 15 minutes. This preincubation step allows the oligo$(dT)_{23}$ primers to be extended by the enhanced AMV-RT before incubating at a temperature between 42-50 °C. The preincubation is recommended for anchored oligo$(dT)_{23}$ primers, but is not required.

6. Place tubes at a temperature between 42-50 °C for 50 minutes.

Note: The optimal reaction temperature should be determined empirically. It is suggested that the reaction be run at a temperature between 42-50 °C initially. Raising the transcription reaction temperature incrementally (up to 65 °C) is recommended for transcribing templates with difficult secondary structure. If the transcription reaction is run at elevated temperatures, a drop in yield may occur.

7. The first strand cDNA is now ready for subsequent PCR amplification, cloning, library synthesis, etc.

References

1. Eastlund, E. and Song, K. (2000) Sigma's New Enhanced Avian RT-PCR Kit. Life Science Quarterly, **1,** 15-17
2. Brooks, E.M., *et al.* (1995) Secondary structure in the 3' UTR of EGF and the choice of reverse transcriptases affect the detection of message diversity by RT-PCR. Biotechniques **19(5):** 806-812
3. Goelet, P., *et al.* (1982) Nucleotide sequence of tobacco mosaic virus RNA. Proc. Natl. Acad. Sci. U.S.A. **79(19):** 5818-5822

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† The PCR process is covered by patents owned by Hoffman-LaRoche, Inc. Purchase of this product does not convey a license under these patents.