Product Specification

Reverse Transcriptase Primers, cDNA Cloning Primers

Oligo d(T) Primers

Shipped at ambient temperature. Store at -20°C
For research use only. Not for use in diagnostic procedures for clinical purposes.
Commercial licenses may be obtained directly from Gene Link.

Item	Lot No.	Catalog No.	Product Description	Size	Quantity		MW
❑		26-4000-02	Oligo d(T)18	18 mer	100 µg	~15 nmols	5413
			Sequence: 5'-TTTTTTTTTTTTTTTTT-3'				
❑		26-4000-05	Oligo d(T)12-18	12-18 mer	100 µg	~20 nmols	4501*
❑		26-4002-10	Oligo d(T)23	23 mer	50 µg	~7.5 nmols	6934
			Sequence: 5'-TTTTTTTTTTTTTTTTTTTTTTT-3'				
❑		26-4002-11	Oligo d(T)23 VN	25 mer	50 µg	~7.5 nmols	7554
			Sequence: 5'-TTTTTTTTTTTTTTTTTTTTTTTVN-3'				
❑		26-4002-16	Oligo d(T)36	36 mer	50 µg	~6 nmols	10889
			Sequence: 5'- TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-3'				
*An average molecular weight is reported.							

Description

Oligo d(T)12-18 is the classic primer mix used to prime synthesis of the first strand cDNA by reverse transcriptase using poly A^+ mRNA as a template.

Oligo dT of various sizes are synthesized individually and gel purified. Oligo d(T)12-18 is a mixture of individually synthesized and purified primers of varying sizes. These are mixed in an equimolar ratio. Oligo d(T)23 VN is particularly suited to initiate reverse transcription adjacent to the start of the poly A tail.

The product is supplied as a lyophilized powder. Oligo purity is greater than 98% as determined by denaturing polyacrylamide gel electrophoresis.

Reconstitution

Recommended reconstitution is at a concentration of 50 µM in RNase-free DEPC treated water.
- Spin the tube briefly to bring down the contents of the tube that may have lodged in the cap during shipment. Pellet may be very small and not visible.

Recommended Reconstitution Protocol			
Catalog No.	Product Description	Volume of RNase-free water to add	Final Concentration
26-4000-02	Oligo d(T)18	300 µl	50 µM (50 pmol/µl; 0.33 µg /µl)
26-4000-05	Oligo d(T)12-18	400 µl	50 µM (50 pmol/µl; 0.25 µg /µl)
26-4002-10	Oligo d(T)23	150 µl	50 µM (50 pmol/µl; 0.33 µg /µl)
26-4002-11	Oligo d(T)23 VN	150 µl	50 µM (50 pmol/µl; 0.33 µg /µl)
26-4002-16	Oligo d(T)36	120 µl	50 µM (50 pmol/µl; 0.41 µg /µl)
Store at -20°C or below after reconstitution.			

Recommended Usage

Use 2 µl of the 50 µM solution for 1 µg poly $(A)^+$ RNA as a template in a 20 µl reaction volume.
See reaction conditions for more details.

Quality Control Data

This product is certified to prime first strand cDNA reaction using poly $(A)^+$ RNA as a template.

Functional Assay Conditions

The conditions given below have been tested to yield first strand cDNA synthesis and is given as an example. Variations and other protocols have been used by other laboratories using this product to yield excellent first strand synthesis. Investigators can substitute their own reaction conditions.

The quality of RNA is very important for the reverse transcription reaction. It is essential to have intact full length RNA as the template material that is free of even trace amounts of RNases and contaminating chemicals. Poor quality RNA template is usually the cause of truncated and incomplete cDNA products.

Add components in the order given below. Reaction volume can be scaled up.

Component	Volume	Comments
poly$(A)^+$ RNA in sterile water Quantity ~1.0 µg	up to 10 µl	Use RNAse free reagents and disposables.
RNase-free water	variable	Calculate total volume and add appropriate volume of RNase-free water at this stage.
50 µM oligo(dT) primer solution	2 µl	Final concentration is 5 µM (5 pmol/ µl).
Heat mixture to 70°C for 10 min, and quick chill on ice.		
5X first strand buffer [250 mM Tris-HCl (pH 8.3), 375 mM KCl, 15 mM MgCl2]	4 µl	
0.1 M DTT	2 µl	
dNTPs (5 mM each dNTP)	2 µl	Final concentration is 0.5 mM of each dNTP.
[α-^{32}P]dCTP (1 µCi/µl)	1 µl	Tracer optional. Add only if required.
Reverse transcriptase; 200 units	1- 2 µl	
Total Volume	**20 µl**	
Incubate at 37°C for 1 hour.		

Related Products

Gene Link stocks various oligo dT primers, oligo dTVN primer, Oligo dT T7 primer, random primers, including an array of fluorescent dye labeled primers for genetic analysis using florescent detecting instruments. The C-12 amino labeled primers are ready to be conjugated to the investigators choice of NHS-activated ligand.

Random Primers are a mixture of oligonucleotides representing all possible sequence for that size. Random Primers can be used to prime synthesis in oligo-labeling similar to using hexamers (1,2) and cDNA synthesis. Random prime labeling yields high specific activity labeled DNA probe which can be used for all southern, northern and in situ hybridization studies. Random Primers can be also used similar to using hexamers in cDNA synthesis in combination with oligo dT to yield more 5′ end cDNA sequence.