Product Specification

Catalog No. 26-4000-XX
Random Primers Phosphorylated

Random Primers 5' Phosphorylated

For research use only. Not for use in diagnostic procedures for clinical purposes. Commercial licenses may be obtained directly from Gene Link.

Quantity	**50 µg**
Shipping Condition	**Ambient**
Storage	**-20°C**

Item	Lot No.	Catalog No.	Product Description	5' - Mod	Size	nmol	MW*
❑		26-4000-10	Random Hexamer phosphorylated; pd(N)6	Phosphate	6 mer	~26	1872
❑		26-4000-07	Random Heptamer Phosphorylated; pd(N)7	Phosphate	7 mer	~22	2181
❑		26-4000-08	Random Octamer Phosphorylated; pd(N)8	Phosphate	8 mer	~20	2490
❑		26-4000-09	Random Nonamer Phosphorylated; pd(N)9	Phosphate	9 mer	~17	2799
*An average molecular weight is reported.							

Description

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 d(T) to yield more 5' end cDNA sequence.

Recently random primers have been used to detect DNA polymorphism. These polymorphisms, simply detected as DNA segments which amplify from one parent but not the other, are inherited in a Mendelian fashion and can be used to construct genetic maps in a variety of species. The authors suggested that these polymorphisms be called RAPD (pronounced RAPID) makers, after Random Amplified Polymorphic DNA (3).

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
26-4000-10	Random Hexamer phosphorylated; pd(N)6	520 µl	50 µM (50 pmol/µl; 0.09 µg /µl)
26-4000-07	Random Heptamer Phosphorylated; pd(N)7	440 µl	50 µM (50 pmol/µl; 0.11 µg /µl)
26-4000-08	Random Octamer Phosphorylated; pd(N)8	400 µl	50 µM (50 pmol/µl; 0.13 µg /µl)
26-4000-09	Random Nonamer Phosphorylated; pd(N)9	340 µl	50 µM (50 pmol/µl; 0.15 µg /µl)
Store at -20°C or below after reconstitution.			

Recommended Usage
Use 4 µl of the 50 µM solution for 1 µg DNA or 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 reverse transcriptase and poly $(A)^+$ RNA as a template, and probe labeling in random prime labeling reactions using klenow DNA polymerase.

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 Random Primer solution	4 µl	Final concentration is 10 µM (10 pmol/ µl).
50 µM oligo(dT)12-18 primer solution (50 pmol/µl = ~0.5 µg/µl)	1 µl	Final concentration is 2.5 µM (2.5 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.

References

1. Feinberg, A.P. & Vogelstein, B. (1983) Anal. Biochem. 132:6-13.

2. Feinberg, A.P. & Vogelstein, B. (1984) Anal. Biochem. 137:266-267.

3. Williams J. G., Kubelik A.R., Livak K.J., Rafalski J.A. & Tingey S.V. (1990) Nucleic Acid Res. 18(22):6531-5.