PERKINELMER™ LIFE SCIENCES, INC.

OLIGONUCLEOTIDE 5' END LABELING SYSTEM

Catalog Number NEP101

For Laboratory Use Only
Caution: Research chemicals for research purposes only.

Contains reagents for 5' end labeling oligonucleotides.

Note: Tracer must be ordered separately.

I. INTRODUCTION

The techniques for end labeling oligonucleotides with radioisotopes have allowed for rapid developments in nucleic acid probe technology. Oligonucleotide probes can be custom made based on sequence information of the target DNA or RNA in several hours on a DNA synthesizer. This eliminates the usual cumbersome and time consuming steps that are involved in the cloning and isolation of restriction fragments to be used as hybridization probes. Another advantage of oligonucleotide probes is that they can be designed to detect single base changes (mutations) in a gene[1-5]. This is an especially useful property, by adjusting the conditions of the hybridization and washes (high stringency), only probes with a perfect match can be made to hybridize. Several techniques have been developed which allow single base changes to be detected using these DNA or RNA probes[6,7].

Synthetic oligonucleotides are prepared with free 5' and 3' hydroxyl groups. This means there is no need for pretreatment with bacterial alkaline or calf intestinal phosphatase. End labeling of oligonucleotides is also much simpler since there is no need to modify reaction conditions depending upon whether the fragment has 5' or 3' overhangs or blunt ends as with restriction fragments[8]. Oligonucleotides can be labeled at either the 3' or 5' end. Using polynucleotide kinase and ATP ^{32}P (BLU/NEG502Z) the 5' end is labeled. Using terminal transferase and deoxynucleotide *5'* triphosphates the 3' end is labeled. The isotope of choice has been ^{32}P, however ^{35}S and ^{125}I have been used successfully[9]. Oligonucleotides labeled with ^{32}P are reported to be stable for 1-2 weeks and those labeled with ^{35}S last approximately 6 times longer. The stability of 125I probes is currently under evaluation. The ^{35}S or ^{125}I labeled oligonucleotides are especially useful when high resolution (as in *in situ* hybridization) or long probe stability is needed.

II. PRINCIPLE OF OLIGONUCLEOTIDE 5' END LABELING

Polynucleotide kinase, simultaneously identified by Richardson[10], and Novagrodsky and Hurwitz[11] in T-phage infected *E. coli*, catalyzes the transfer of the gamma phosphate of ATP to the 5' hydroxyl terminus of DNA or oligonucleotide molecules. Oligonucleotides can be labeled with radioactivity if the gamma phosphate of ATP contains phosphorous-32. Labeling the 5' ends with ^{35}S utilizing ATP (gamma thiophosphate), [^{35}S] is not recommended due to the slow rate of the enzyme with ATPαS as substrate[12]. Complete phosphorylation of the 5' hydroxyls is achieved using low concentrations of ATP, usually a two to five-fold excess over the concentration of 5' hydroxyl ends.

III. EXPLANATION OF THE SYSTEM

PerkinElmer Life Sciences, Inc. End Labeling System is an adaptation of the procedure described by Maniatis[13], which is optimized to obtain maximal labeling of the oligonucleotide. The system includes buffers, enzyme, a control oligonucleotide and the other reagents for labeling the 5' termini of oligonucleotides. To check that the system is functioning properly, a 17 mer oligonucleotide has been included as a control. The T_4-polynucleotide kinase has been purified to homogeneity, as demonstrated by SDS. gel electrophoresis, and has been shown to be free of any detectable contaminating ribonuclease, deoxyribonuclease, or phosphatase activities.*

T_4-Polynucleotide Kinase, NEE101.

IV. THE FOLLOWING REAGENTS ARE SUPPLIED IN THE KIT

Store kit components at temperature indicated in this section, in general, all enzymes and reagents at -20°C. Enough of each reagent is supplied with this kit for minimally 10 labeling reactions.

T_4 Polynucleotide Kinase (NEE101)

One vial containing 30 ul (30 units*) of polynucleotide kinase (EC2.7.1.78) from T_4-infected *E. coli* is provided already diluted to the proper concentration for use (1000 units/ml). The enzyme has been purified to homogeneity and is stocked in Tris-HCl buffer, pH7.6, containing ATP, DTT, KCI, EDTA, and 50% glycerol. The material is stable for at least one month when stored at -20°C.

Phosphorylation Buffer

One vial containing 0.15 ml of a l0x buffer is supplied for the phosphorylation reaction. The buffer is a solution of Tris-HCI, pH7.6, $MgCl_2$, DTT, and spermidine. The material is stable for at least one month when stored at -20°C.

Control Oligonucleotide

Approximately 50 pmole of a 17 mer control oligonucleotide is supplied in 50 µl of deionized water. The preparation is supplied ready to use as a control to check the system according to the protocol provided. The material is stable at least one month when stored at -20°C.

Deionized Water

One vial containing 2 mL deionized water is supplied. This component is provided to minimize interfering substances (e.g. metals, ions, and

enzymes) present in some water supplies, which might inhibit the enzymatic reactions.

V. SUPPLIES AND EQUIPMENT NEEDED

Vortex mixer
Pipetting equipment
Water bath for 37°C incubations
Plastic gloves
Polypropylene microcentrifuge tubes, or siliconized glass
Brinkman PEI cellulose thin layer strips (optional)
3 or 5 cc disposable syringes
Microcentrifuge
Methanol
20% ethanol
Water
Isotope

VI. REACTION PROTOCOLS FOR LABELING 5' END LABELING

Described below is the recommended procedure for 5' end labeling oligonucleotides with phosphorous-32. The 17mer control oligonucleotide supplied with the kit is used as a control for the labeling procedure.

A. Preparation of Oligonucleotide for End Labeling

Prior to the end labeling, it is recommended that the oligonucleotide be purified to remove any contaminating salts, reagents, organic solvents, or proteins which might affect the reaction. The oligonucleotide may be purified by a wide variety of standard procedures, including HPLC or purify by method of choice. After purification, the oligonucleotide should be taken to dryness (nitrogen stream or under vacuum).

B. 5' End Labeling Reaction

1. The procedure is written for 10 pmoles of oligonucleotide. The material may be in any volume up to 25 µl of water or buffer, pH 7.6.

2. Add 5 µl of phosphorylation buffer (10x) to the tube containing 10 pmoles of oligonucleotide.

3. Add deionized water so that the final reaction volume will be 50 µl.

4. Add tracer to the reaction tube. The number of

picomoles of ATP, [γ-^{32}P], should be at least 2.0 times the number of picomoles of 5' ends. For labeling 10 pmoles of oligonucleotides, 20 pmoles of ^{32}P-ATP is needed or approximately 10 µl of ATP, [γ-^{32}P], (BLU/NEG502Z), solution is recommended. Mix the contents well and centrifuge briefly.

5. Add 3 µl, (3 units) of polynucleotide kinase solution. Mix the solution gently. Spin down briefly in a microcentrifuge.

6. Incubate at 37°C for 30 minutes.

7. The reaction may be followed analytically as follows. Spot small aliquots (0.1 µl volume, the smaller volume the better) on a PEI-Cellulose thin layer strip (Brinkman). The TLC is developed in 1M KH_2P0_4 buffer for 20 minutes. The buffer in the chamber should not reach the application spot. Labeled oligonucleotide remains at the origin and ATP, [^{32}P] migrates with an Rf of approximately 0.6-0.7. The TLC strip is either autoradiographed or scanned with a GM-tube.

8. To stop the reaction add 5 µl of 250 mM EDTA and cool to 4°C on ice.

5' END LABELING REACTION SUMMARY

	Vol. (µL)
Oligonucleotide (10 pmol)*	X = 5-25
1Ox Phosphorylation Buffer	5
ATP, [γ-^{32}P], (100 uci)	10
Water	32 - X 22-2
Mix and spin down	
Add Polynucleotide Kinase	3
	50 µl

Incubate at 37°C for 30 minutes. Stop the reaction with 5 µl of 0.1M EDTA pH 8.0. Place on ice.

*1 O.D. = 20 µg/mL, average molecular weight dNMP = 325[13]. Purify by method of choice.

VII. REFERENCES

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