



BioProbe®
3'-Oligonucleotide Labeling Kit
with Fluorescein-12-ddUTP

Cat. No. 42730-33
[25 labeling reactions]

For Research Use Only

INTRODUCTION

Terminal labeling is an ideal procedure for the labeling of oligonucleotides with haptens such as biotin or fluorescein. An oligonucleotide can be synthesized using standard, commercially available reagents and labeled after synthesis in a simple and reproducible enzyme reaction.

The ENZO **BioProbe® 3'-Oligonucleotide Labeling Kit with Fluorescein-12-ddUTP** contains all reagents required for terminal labeling of oligonucleotides with fluorescein except the oligonucleotides to be labeled. The ENZO **BioProbe® 3'-Oligonucleotide Labeling Kit** provides maximum flexibility for labeling oligonucleotides approximately 15 to 100 nucleotides in length. A standard reaction will label up to 100 picomoles (1 µg of a 30-mer oligonucleotide sequence).

A complete kit contains two component packs that are available separately: a Reagent Pack and a Fluorescein-12-ddUTP Dideoxynucleotide Pack. Both packs contain sufficient reagents for 25 labeling reactions.

KIT REAGENTS PROVIDED

A. **BioProbe® 3'-Oligonucleotide Labeling System Reagent Pack** [25 reactions] contains:

5X Reaction Buffer, 100 µl
Potassium cacodylate buffer, pH 7, containing β-mercaptoethanol

10X CoCl₂ Solution, 50 µl
10mM solution

Terminal Deoxynucleotide Transferase, 50 µl
30 units/µl in storage buffer

Control Oligonucleotide (unlabeled), 25 µl
0.2 µg/µl in TE Buffer (30-mer, 5'-TTG GGT AAC GCC AGG GTT TTC CCA GTC ACG-3', homologous to the *lac Z'* region of pUC-type vector DNAs)

Control Target DNA, 25 µl
0.2 µg/µl in TE Buffer (Restriction enzyme digest of a pUC-type vector containing an Epstein-Barr Virus DNA insert)

WARNING: *The 5X Reaction Buffer and Terminal Deoxynucleotide Transferase contain potassium cacodylate which contains arsenic. It is toxic. Use gloves during the handling of these reagents. Dispose of these reagents and the individual labeling reaction waste materials according to local regulations.*

B. **Fluorescein-12-ddUTP Dideoxynucleotide Pack for 3'-Oligonucleotide Labeling** [25 reactions] contains:

Fluorescein-12-ddUTP, 25 µl
1 mM Fluorescein-12-ddUTP

Labeled Control Oligonucleotide, 20 µl
0.05 µg/µl in TE Buffer (Fluorescein-12-ddUTP labeled *Lac Z'*-specific sequence identical to the unlabeled control oligonucleotide in the Reagent Pack)

EQUIPMENT AND REAGENTS REQUIRED BUT NOT PROVIDED

Preparation and Analysis of Labeled Oligonucleotide Probes

- 37°C Water Bath
- Ethidium Bromide
- Agarose
- UV Transilluminator

Termination of Labeling Reaction

- 0.2 M EDTA, pH 8

Purification of Oligonucleotide Probes

- 4M LiCl
- Prechilled (-20°C) Ethanol
- TE Buffer
- Microcentrifuge

STORAGE

1. Upon receipt, store all reagents at -20°C, in a freezer that is not self defrosting.
2. After initial use, store the unlabeled and labeled control oligonucleotides at 2-8°C.
3. After initial use, continue to store all other reagents at -20°C.

This product or the use of this product is covered by one or more claims of Enzo patents, including, but not limited to, the following: U.S. Patent No. 4,994,373; EP 0 329 198 B1; EP 0 063 879 B1; DK 171 822; Canadian Patent No. 1,309,672; Japanese Patent Nos. 2,131,226 and 1,416,584 and patents pending.

PROCEDURES FOR LABELING OF OLIGONUCLEOTIDES**3'-Oligonucleotide Labeling Reactions**

1. Add the following reagents (in the indicated order) to a microcentrifuge tube, keeping the tube at **room temperature** while additions are made:

Reagent	Volume
5X Reaction Buffer	4 µl
10X CoCl ₂	2 µl
Oligonucleotide to be labeled	variable (to give 1 µg of 30-mer or 100 pmol); use 5 µl for control oligonucleotide
Fluorescein-12-ddUTP Dideoxynucleotide Pack	1 µl
Terminal Deoxynucleotide Transferase	2 µl
Distilled or deionized water	variable (to give a final reaction volume of 20 µl)
Total Volume	20 µl

2. Carefully mix the reagents in the tube and collect the mixture in the bottom of the microcentrifuge tube by brief (5 second) microcentrifugation.
3. Incubate the tube for **15 minutes** in a 37°C water bath. The reaction may be allowed to proceed for up to 1 hour.
4. If desired, transfer the tube to ice water (2-4°C), remove 2 µl and analyze on an agarose or acrylamide gel.
5. Stop the reaction by adding 5 µl of 0.2 M EDTA.
6. Store the labeled oligonucleotide probe at -20°C. Do not freeze and thaw repeatedly.

PURIFICATION OF BIOTIN AND FLUORESCHEIN-LABELED OLIGONUCLEOTIDE PROBES

For most membrane applications the labeled oligonucleotides can be used without further manipulation because unincorporated nucleotides do not adhere to the membrane and are washed away during detection. If purification is desired, the following protocol may be used.

1. Add 4 µl of 4M LiCl and 75 µl of prechilled (-20°C) ethanol. Mix well.
2. Leave for 30 minutes at -70°C or 2 hours at -20°C.
3. Centrifuge at high speed (13,000 x g) for 10-15 minutes.
4. Remove and discard supernatant, being careful not to disturb the pellet.
5. Wash the pellet with 100 µl of prechilled (-20°C) ethanol, vortex to mix and then centrifuge for 10-15 minutes.
6. Remove and discard supernatant, being careful not to disturb the pellet.
7. Dry the oligonucleotide probe pellet briefly under vacuum and dissolve in small volume of TE buffer to the desired concentration.
8. Store the labeled probe at -20°C or -70°C. Probes are stable for several years when stored frozen.

REFERENCES

1. Brakel, C.L. and Engelhardt, D.L. [1984] in Symposium on Rapid Detection and Identification of Infectious Agents, Kingsbury, D. T. and Falkow, S. (eds), Academic Press, N.Y., pp. 235-243.
2. Cook, A. F., Vuocolo, E. A., and Brakel, C. L. [1988] Nucleic Acids Research, 16:4077-4095.

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Rev. 4.1 August 2007