BioProbe®
3'-Oligonucleotide Labeling System

Reagent Pack
[25 labeling reactions]

Cat. No. 42730

For use with
***BioProbe*® 3'-Oligonucleotide Tailing System Deoxynucleotide Packs:**
Cat. No. 42741

***BioProbe*® 3'-Oligonucleotide Labeling System Dideoxynucleotide Packs:**
Cat. Nos. 42731 and 42733

For Research Use Only

INTRODUCTION

The procedure for labeling of DNA probes with a polynucleotide "tail" containing hapten-labeled nucleotides was developed by Enzo.[1,2] In such terminal labeling reactions, terminal transferase catalyzes the addition of nucleotides to any 3'-OH terminus in a template independent manner. This rapid and convenient nonradioactive labeling procedure is free of any sequence bias that is normally observed in random priming or nick translation reactions.

Terminal labeling is an ideal procedure for the labeling of oligonucleotides. 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 System** 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 system combines a standard Reagent Pack (buffers and enzyme) with a Nucleotide Pack. All components contain sufficient reagents for 25 labeling reactions.

The system is available in two modular formats:

3'-Oligonucleotide Tailing (with a "tail" of nucleotides)

3'-Oligonucleotide Labeling (with a single nucleotide)

The ENZO ***BioProbe*® 3'-Oligonucleotide Labeling System Reagent Pack** contains all reagents required for labeling except nucleotides. For optimun results, the reagents contained in this Pack should be used with the appropriate **Nucleotide Pack**.

REAGENTS PROVIDED

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

10X $CoCl_2$ 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.*

ADDITIONAL REAGENTS REQUIRED
(Available separately or combined with Cat. No. 42730)

ENZO *BioProbe*® 3'-Oligonucleotide Tailing System Deoxynucleotide Pack:

- Modified Deoxynucleotide, 50 μl
  0.5mM modified dNTP (1mM for Fluorescein-12-dUTP)
- dATP, 50 μl
  5 mM solution
- Labeled Control Oligonucleotide, 20 μl
  0.05 μg/μl in TE Buffer (*Lac Z'*-specific sequence identical to the unlabeled control oligonucleotide, labeled with the specific deoxynucleotide of the Deoxynucleotide Pack using standard labeling conditions)

ENZO *BioProbe*® 3'-Oligonucleotide Labeling System Dideoxynucleotide Pack:

- Modified Dideoxynucleotide, 25 μl
  1 mM modified ddNTP
- Labeled Control Oligonucleotide, 20 μl
  0.05 μg/μl in TE Buffer (*Lac Z'*-specific sequence identical to the unlabeled control oligonucleotide, labeled with the specific dideoxynucleotide of the Dideoxynucleotide Pack using standard labeling conditions)

EQUIPMENT AND REAGENTS REQUIRED BUT NOT PROVIDED

Preparation and Analysis of Labeled Oligonucleotide Probes

- 37°C Water Bath
- Agarose
- Ethidium Bromide
- 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.

PROCEDURES FOR TAILING AND LABELING OF OLIGONUCLEOTIDES

A. 3'-Oligonucleotide Tailing 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
5mM dATP (from Deoxynucleotide Pack)	2 µl
Modified Deoxynucleotide (from Deoxynucleotide Pack)	2 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. If longer tail is desired, 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.

B. 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
Modified Dideoxynucleotide (from 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 FLUORESCEIN-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 prechillled (-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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