BioProbe®
Nick Translation DNA Labeling System

Reagent Pack
[25 labeling reactions of 1 μg DNA]

Cat. No. 42710

For use with Nick Translation DNA Labeling System Deoxynucleotide Packs: Cat. Nos. 42712 and 42716

For Research Use Only

INTRODUCTION

Nick translation (Rigby, P. W. J., et al. [1977] J. Mol. Biol. 113:237) is based on the concerted action of *E. coli* DNA Polymerase I and pancreatic DNase I. DNase I introduces random scissions, or "nicks", in DNA and *E. coli* DNA Polymerase I catalyzes sequential addition of nucleotide residues to the 3'-OH terminus while simultaneously eliminating nucleotides from the 5'-PO_4 terminus of the nicks. There is no net DNA synthesis. As new incorporation occurs at the 3' terminus, nucleotides are removed from the 5' terminus resulting in movement, *i.e.,* translation, of the nick linearly along the DNA strand. In the presence of modified nucleotides, pre-existing unmodified nucleotides are replaced by the analog nucleotides.

Nick translation is recommended for labeling of double stranded DNA that is larger than 1kb. Nick translated probes are excellent for use in both *in situ* and membrane hybridization applications. Following hybridization with a nick translated probe carrying a chemical modification, the DNA probe can be detected by its interaction with an appropriate hapten-binding protein that is labeled with a fluorescent dye or a color producing enzyme.

The ENZO ***BioProbe***® **Nick Translation DNA Labeling System** provides the researcher with a simple method for preparing a variety of hapten-labeled probes. A complete system consists of the combination of two separate components: a Reagent Pack and one of two different Deoxynucleotide Packs. The Reagent Pack contains all of the reagents (except deoxynucleotides) required to carry out nick translation reactions, including DNase I and DNA Polymerase I. The Nick Translation System Reagent Pack also contains a vial of concentrated DNase I Dilution Buffer, a vial of Stop Buffer for terminating the nick translation reactions and a vial of unlabeled Control Template DNA.

The Reagent Pack can be purchased separately or in combination with a Deoxynucleotide Pack. The two different Deoxynucleotide Packs, which are also available separately, contain the modified deoxynucleotide of choice (modifications include Biotin and Fluorescein) in a ready-to-use form with the optimized concentration of the other deoxynucleoside triphosphates. The Deoxynucleotide Packs also contain a vial of Labeled Control DNA that has been labeled with the analog nucleotide contained in the specific deoxynucleotide mixture.

Because the methods for labeling DNA with the ENZO ***BioProbe***® **Nick Translation DNA Labeling System** are consistent for all of the haptens, labeling reactions using different analog nucleotides and different DNAs can be carried out at the same time with the same reaction conditions. Thus, combination of the Nick Translation Reagent Pack with two different Nick Translation Deoxynucleotide Packs provides a simple, convenient and economical way to label DNA probes with two different haptens.

Each Reagent and Deoxynucleotide Pack contains sufficient amounts of reagents for labeling 25 μg of DNA template.

REAGENTS PROVIDED

DNase I, 50 μl
0.5 mg/ml in 0.1M $MgCl_2$

50X DNase I Dilution Buffer, 1 ml
0.5 mg/ml BSA in 2.5M $MgCl_2$

DNA Polymerase I, 125 μl
1 unit/μl in storage buffer

Stop Buffer, 125 μl
0.2M EDTA

Control Template DNA, 25 μl
0.2 μg/μl in TE Buffer (10mM Tris Buffer, pH 8.0, 1mM EDTA)

ADDITIONAL REAGENTS REQUIRED
(Available separately or combined with Cat. No. 42710)

Nick Translation Deoxynucleotide Pack:

- Deoxynucleotide Mix, 125 μl
  dATP, dCTP, dGTP, TTP and modified dNTP in Tris buffer
- Labeled Control DNA, 25 μl
  40 μg/ml in TE Buffer

EQUIPMENT AND REAGENTS REQUIRED BUT NOT PROVIDED

Preparation and Analysis of Nick Translated Probes

- Water Bath set to 15°C
- Agarose
- Ethidium Bromide
- UV Transilluminator

Purification of Nick Translated Probes

- 10% SDS solution
- TE Buffer
- TE Buffer-Saturated Phenol
- Chloroform
- 3M NaAcetate Buffer, pH 4.8
- 95% Ethanol
- 70% Ethanol
- 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 DNase I in 5-10 μl aliquots at -20°C (avoid repeated freezing and thawing of the DNase I stock solution).
3. After initial use, continue to store reagents at -20°C.

NICK TRANSLATION LABELING OF DNA

A. Dilution of DNase I

1. The DNase I provided in this kit is titered for use at a dilutions ranging from 300- to 1000-fold. Use a dilution of 500-fold for plasmid DNAs containing inserts of over 4 kb and 800-fold for plasmid DNAs containing inserts of less than 4 kb. (If the DNA is very large, use a 300-fold dilution; if the DNA is very small, use a 1000-fold dilution.)
2. Prepare 1X DNase I Dilution Buffer: Mix 980 μl of high quality distilled or deionized water with 20 μl of 50X DNase I Dilution Buffer, and keep on ice.
3. When all **other** reaction components have been added to the reaction tubes, see below, prepare the required dilution of DNase I using the 1X Dilution Buffer. Add the freshly diluted DNase I to otherwise complete reaction tubes.

When used with modified deoxynucleotides, 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; Canadian Patent No. 1,309,672; and patents pending.

B. Nick Translation Reaction

1. Add reaction components to clean microcentrifuge tubes that are kept on ice while additions are made. Make additions in the order indicated in the following table.
   For increased amounts of labeled DNA, up to 5 μg per 50 μl reaction can be labeled by increasing the amount of template DNA and proportionately increasing the amount of deoxynucleotide mix without increasing the amount of DNA Polymerase I or DNase I. See scaled up synthesis.

Reagent	Standard Synthesis	Scaled Up Synthesis
DNA template	variable volume to obtain 1 μg DNA (or 5 μl of Control Template DNA)	variable volume* (up to 5 μg of DNA)
Nick Translation Deoxynucleotide Mix (From Nick Translation Deoxynucleotide Pack)	5 μl	**up to 25 μl***
Distilled or deionized water	variable (to bring final reaction volume to 50 μl)	variable (to bring final reaction volume to 50 μl)
DNA Polymerase I	5 μl	5 μl
DNase I (freshly diluted)	5 μl	5 μl
Total Volume	**50 μl**	**50 μl**

Use 5 μl of Nick Translation Deoxynucleotide Mix for each μg of template DNA.

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. Immediately place the tube in a 15°C water bath. Incubate for 90-120 minutes.
4. Remove the tube to ice water (2-4°C). Remove 2 to 5 μl and analyze on a 1.2 to 1.4% agarose gel. If the fragmented DNA ranges in size from 200 to 1000 base pairs, the nick translation reaction was successful.
5. Add 5 μl of Stop Buffer to terminate the reaction. Heat the stopped reaction for 5 minutes at 65°C.

PURIFICATION OF DNA PROBES

For many applications the labeled DNA can be used without further manipulation. If purification is desired, the following protocol may be used.

NOTE: While it has been accepted that chemical labeling creates a DNA that is lost during phenol extraction, we have found that the following procedure results in 70-100% recovery of biotin-, and fluorescein-labeled DNA probes. Follow this protocol exactly as described. It is important to allow the ethanol precipitation step to proceed for 6 hours or more to achieve this level (70-100%) of recovery.

When working with the small volumes of the nick translation reactions (i.e., 50 μl), for convenience and practicality, dilute the reaction mixture with at least 50 μl of TE buffer (10mM Tris buffer, pH 8.0, 1 mM EDTA).

1. Add high quality SDS to give a final concentration of 0.1% (e.g., 1 μl of a 10% sterile SDS solution to a 50 μl reaction that has been diluted with 50 μl of TE). Mix well.
2. Add one volume of a 1:1 mixture of room temperature Phenol (TE Buffer saturated):Chloroform (e.g., 100 μl to the diluted, SDS-treated reaction) and extract by gentle mixing for 2 minutes.
3. Separate the phases by microcentrifugation for 2 minutes **at room temperature.**
4. Collect the aqueous (top) phase into a clean tube and extract with one volume of Chloroform. Again, separate the phases by microcentrifugation for 2 minutes at room temperature.
5. Collect the aqueous (top) phase into a clean tube.
6. Add one-tenth volume 3M sodium acetate, pH 4.8 (i.e., 10 μl in this example). Mix. Add two volumes (200 μl) ice cold 95% ethanol.
7. Store the precipitating material **at least 6 hours at -20ºC** to ensure complete precipitation of the small fragments produced by the labeling reaction.
8. Collect the precipitate by 5 minute centrifugation in the cold.
9. Wash the pellet with ice-cold 70% ethanol.
10. Dry the pellet thoroughly and resuspend in a small volume of TE. Determine the concentration of DNA by spectrophotometry or by comparison in a detection dilution series using the Labeled Control DNA provided in the Deoxynucleotide Pack as a titration standard.
11. Store the labeled probe at -20°C (particularly for fluorescein-labeled probes) or at 4°C. Probes are stable for several years when stored frozen.
12. If desired, G10 or G25 chromatography can be used to remove the small amounts of unincorporated nucleotide that remain in the preparation.

For Technical Assistance call ENZO:
Toll free from the U.S. and Canada: 1-800-221-7705
All others: 631-694-7070
Fax: 631-694-7501
email: enzolifesciences@enzobio.com
http://www.enzolifesciences.com

Rev. 3.1, August 2007

