Invitrogen™
life technologies

Nick Translation System

Cat. No. 18160-010 **Size: 50 Reactions**

Store at -20°C in a non-frost-free freezer.

Description

The Nick Translation System is suitable for both radioactive and nonradioactive labeling of DNA. Five pre-mixed nucleotide solutions are included which allow flexibility in choice of labeled nucleotide.
NOTE: Labeled nucleotide is not included.

Component	Amount
dNTP Mix (minus dATP): 0.2 mM each of dCTP, dGTP, dTTP, 500 mM Tris-HCl (pH 7.8), 50 mM $MgCl_2$, 100 mM 2-mercaptoethanol.	250 µl
dNTP Mix (minus dCTP): 0.2 mM each of dATP, dGTP, dTTP, 500 mM Tris-HCl (pH 7.8), 50 mM $MgCl_2$, 100 mM 2-mercaptoethanol.	250 µl
dNTP Mix (minus dGTP): 0.2 mM each of dATP, dCTP, dTTP, 500 mM Tris-HCl (pH 7.8), 50 mM $MgCl_2$, 100 mM 2-mercaptoethanol.	250 µl
dNTP Mix (minus dTTP): 0.2 mM each of dATP, dCTP, dGTP, 500 mM Tris-HCl (pH 7.8), 50 mM $MgCl_2$, 100 mM 2-mercaptoethanol.	250 µl
dNTP Mix (minus dCTP, dGTP): 0.2 mM each of dATP, dTTP, 500 mM Tris-HCl (pH 7.8), 50 mM $MgCl_2$, 100 mM 2-mercaptoethanol.	250 µl
Control DNA: 5 µg pBR322 DNA in 1 mM EDTA, 10 mM Tris-HCl (pH 8.0), 250 µg/ml	20 µl
Pol I/DNase I Mix: 0.5 U/µl DNA Polymerase I, 0.4 mU/µl DNase I, 50 mM Tris-HCl (pH 7.5), 5 mM Mg-acetate, 0.1 mM PMSF, and 50% (v/v) glycerol, 100 µg/ml nuclease-free BSA.	250 µl
Stop Buffer: 0.5 M EDTA (pH 8.0)	500 µl
Distilled H_2O	2 × 1.25 ml

Quality Control

Using the standard nick translation conditions label incorporation into Control DNA is ≥ 1×10^8 cpm/µg.

Part no. 18160010.pps Rev. date: 02/10/03

Procedure for Labeling DNA by Nick Translation

A. Radioactive Probes

1. Select the radioactively labeled nucleotide to be used (A, C, G or T). For most cases, we recommend using dCTP. If the nucleotide is in a 50% ethanol solution or needs to be concentrated we recommend lyophilization or drying with nitrogen (under a fume hood). The 1.5-ml microcentrifuge tube in which the nucleotide has been dried or concentrated can also be used for the nick translation reaction. Use 162.5 pmol radioactive dNTP per 50-µl reaction (final concentration 3.25 µM).

   NOTE: Substitution of [α-^{32}P]dCTP by the same amount of [α-^{32}P]dATP decreases the specific activity of the labeled product.

2. Add the following reagents to a 1.5-ml microcentrifuge tube placed in ice, then mix briefly:

   5 µl dNTP Mix (select one of the five mixes which contains all dNTPs except those to be used in radioactive form)
   _____ µl solution containing 1 µg test DNA (or 4 µl [1 µg] Control DNA)
   _____ µl Radioactive Nucleotide (if not previously dried in this tube) - for example, 13 µl 10 mCi/ml (800 Ci/mmol)
   _____ µl Distilled water
   45 µl Total volume

3. Add 5 µl Pol I/DNase I Mix. Mix gently but thoroughly. Centrifuge 5 seconds in a microcentrifuge.

4. Incubate at 15°C for 60 minutes.

5. Add 5 µl Stop Buffer.

6. Determine the amount of incorporated radioactivity by precipitating a small aliquot of the reaction mixture with trichloroacetic acid (TCA), or proceed immediately to separating the labeled DNA from unincorporated nucleotides. The latter can be achieved by repeated ethanol precipitation: Add 0.5 volumes of 7.5 M ammonium acetate and 2 volumes of ethanol, vortex, centrifuge at 12,000 × g for 30 minutes, remove supernatant. Repeat once.

B. Biotinylated Probes

Use of the BioNick™ Labeling System (Cat. No. 18247-015) is recommended for preparation of biotinylated probes by nick translation.