invitrogen™
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DNA Polymerase I/DNase I

Cat. No. 18162-016 **Size: 250 units**
Conc.: 0.5 U/µl DNA Polymerase I; **Store at -20°C (not frost-free).**
0.4 mU/µl DNase I

Description:
DNA Polymerase I/DNase I is a mixture of both enzymes used for nick translation of DNA. It may be used to label DNA radioactively or with a biotinylated nucleotide. DNA Polymerase I/DNase I is also a component of the Nick Translation Reagent Kit (Cat No. 18160-010).

Unit Definition:
One unit of DNA Polymerase I in the absence of DNase I incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 min at 37°C.

Storage Buffer:
50 mM Tris-HCl (pH 7.5)
5 mM magnesium acetate
1 mM 2-mercaptoethanol
0.1 mM PMSF
100 µg/ml BSA
50% (v/v) glycerol

Quality Control:
This product has passed the following quality control assay: Performance in a nick translation reaction.

Doc. Rev.: 122701

Nick Translation Labeling Protocol:

1. Preparation of radioactive nucleotide:
   Select radioactively labeled nucleotide to be used (A, C, G or T). For most cases, we recommend using dCTP. If nucleotide is in a 50% ethanol solution or needs to be concentrated, we recommend lyophilization or blowing nitrogen on the surface of the solution (under a fume hood). The vial (1.5-ml microcentrifuge tube) in which the nucleotide has been dried down/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).
2. Into a 1.5-ml microcentrifuge tube (sitting in ice) pipet:

5 µl	10X Buffer [500 mM Tris-HCl (pH 7.8), 50 mM $MgCl_2$, 100 mM 2-mercaptoethanol, 100 µg/ml nuclease-free BSA]
5 µl	dNTP Mix [200 µM of each nucleotide **except** the one which is to be used in radioactive form]
X µl	1 µg DNA
Y µl	162.5 pmol radioactive nucleotide (for example, 13 µl 10 mCi/ml (800 Ci/mmol) [α-^{32}P] dCTP)
Z µl	distilled H_2O
45 µl	Total

   Mix briefly.

3. Add 5 µl of DNA Polymerase/DNase I. Close the lid of the tube. Mix gently but thoroughly. Centrifuge briefly. (Microcentrifuge at 15,000 × *g* for 5 seconds).
4. Incubate at 15°C for 60 minutes.
5. Add 5 µl Stop Buffer l [0.5 M EDTA (pH 8.0)]
6. Determine the amount of radioactivity incorporated by precipitating a small aliquot of the reaction mixture in trichloroacetic acid (TCA), or proceed immediately to separating the labeled DNA from nucleotides. The latter can be achieved by chromatography on a 0.9 x 15 cm column of Sephadex® G-50 Fine, equilibrated with 10 mM Tris-HCl (pH 8.0), 50 mM NaCl, 0.1 mM EDTA (pH 8.0) or by repeated ethanol precipitation (add ½ volume of 7.5 M NH_4OAc and 2 volumes of ethanol; repeat once).

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