BioNick™ Labeling System

Cat. No.: 18247-015 **Size: 50 Reactions**
Store at -20°C in a non-frost-free freezer.

Description

The BioNick™ Labeling System is designed for generating small (50–500 base) biotin-labeled DNA probes by nick translation. Small probes are important for successful *in situ* hybridization, and can help to decrease background in filter-based hybridization.

Component		Amount
10X dNTP Mix	0.2 mM each dCTP, dGTP, dTTP 0.1 mM dATP 0.1 mM biotin-14-dATP 500 mM Tris-HCl (pH 7.8) 50 mM $MgCl_2$ 100 mM β-mercaptoethanol 100 µg/ml nuclease-free BSA	250 µl
10X Enzyme Mix	0.5 U/µl DNA Polymerase I 0.007 U/µl DNase I 50 mM Tris-HCl (pH 7.5) 5 mM magnesium chloride 0.1 mM phenylmethylsulfonyl fluoride 50% (v/v) glycerol 100 µg/ml nuclease-free BSA	250 µl
Control DNA	pBR322, 250 µg/ml in 10 mM Tris-HCl (pH 8.0) 0.1 mM EDTA, 5 mM NaCl	20 µl
Stop Buffer	0.5 M EDTA (pH 8.0)	500 µl
Distilled H_2O		2 × 1.25 ml

Quality Control

1. *E. coli* DNA Polymerase I has no detectable double-strand specific endonuclease contamination, and exhibits DNase I-dependent nick translation on intact DNA.
2. Using the standard reaction conditions (over), and control pBR322 plasmid DNA, the sizes of the nick translation products are between 50 and 500 bases.

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For technical questions about this product, call the Invitrogen TECH-LINE℠ 800 955 6288

Labeling Protocol

1. Pipet the following components into a 1.5-ml microcentrifuge tube on ice:

   5 µl 10X dNTP Mix
   __µl (1 µg) DNA
   __µl distilled water to 45 µl
   5 µl 10X Enzyme Mix

   If desired, the standard 1-µg reaction can be increased linearly for large-scale probe production.

2. Close the lid of the tube, mix well and centrifuge briefly (15,000 × *g* for 5 sec).

3. Incubate at 16°C for 1 h.

   NOTE: For *in situ* hybridization applications where a smaller size range of probe is required to facilitate penetration into the sample, the reaction incubation can be extended to 2 h.

4. Add 5 µl Stop Buffer.

5. Separate the unincorporated nucleotides from the labeled DNA probe by repeated ethanol precipitation. Add 1/10 volume 3 M sodium acetate and 2 volumes cold (-20°C) 95% (or absolute) ethanol to the reaction tube. Mix by inverting the tube. Freeze at -70°C (dry ice) for 15 min or at -20°C for 2 h. Centrifuge at 15,000 × *g* for 10 min. Carefully remove the supernatant with an automatic pipettor and dry the pellet. Resuspend the pellet in 50 µl autoclaved H_2O and precipitate the probe with sodium acetate and ethanol as described above. Resuspend the probe in TE buffer [10 mM Tris-HCl (pH 7.5), 1 mM EDTA] and store at -20°C.

6. If probes are to be used for *in situ* hybridization, separate the unincorporated nucleotides from the labeled DNA probe using a column.

7. Biotinylated probes are very stable and can be stored at -20°C in TE buffer for at least one year.