

## **Biotin DecaLabel™ DNA Labeling Kit** (#K0651, for 10 reactions)

The Biotin DecaLabel™ DNA Labeling Kit is based on a random-oligonucleotide DNA labeling method, developed by Feinberg and Vogelstein [1, 2]. The method relies on priming of the polymerase reaction on the template DNA with random decanucleotide primers. The complementary strand is synthesized from the 3'-end of the primer using Large Fragment of DNA Polymerase I, Exonuclease Minus (Klenow Fragment, *exo*<sup>-</sup>) in the presence of nucleoside triphosphates, one of which is labeled with biotin. Biotin-11-dUTP is incorporated into the newly synthesized complementary DNA strand. Using Klenow Fragment, *exo*<sup>-</sup> the reaction time can be prolonged without labeled probe degradation.

The labeling protocol provided can be used for preparation of biotinylated probes using various DNA templates: linearized plasmids, cosmids, supercoiled DNA, DNA fragments.

Biotin-labeled DNA can be detected using any biotin-avidin or biotin-streptavidin detection system.

Probes labeled with Biotin DecaLabel™ DNA Labeling kit can be used for various hybridization techniques: dot/slot, Southern, Northern blots, plaque and colony screening, *in situ* hybridization.

## **COMPONENTS OF THE KIT**

- 1. Klenow Fragment, *exo*<sup>-</sup>, 5u\*/μl:**  
15μl of the enzyme solution in buffer containing 50% glycerol.
- 2. Decanucleotide in 5x Reaction Buffer:**  
100μl of 0.25M Tris-HCl buffer (pH 8.0 at 20°C) containing 25mM MgCl<sub>2</sub>, 5mM dithiothreitol and Random (decamer) primer (12.50.u./ml).
- 3. Biotin Labeling Mix:**  
50μl of 1mM dGTP, 1mM dATP, 1mM dCTP, 0.65mM dTTP, 0.35mM Biotin-11-dUTP aqueous solution.
- 4. Control Template:**  
50μl of λ DNA/HindIII fragments (10ng/μl).
- 5. Biotin-labeled DNA :**  
50μl of biotin-labeled DNA (λ DNA/HindIII), (5ng/μl)
- 6. Water, nuclease-free:**  
1.5ml of 0.22μm membrane-filtered molecular biology grade water.

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*\*One unit of Klenow fragment, *exo*<sup>-</sup>, catalyzes incorporation of 10nmoles of deoxyribonucleotides into DE-81 adsorbable form in 30min at 37°C using poly(dA-dT)•poly(dA-dT) as a template-primer.*

### DNA LABELING WITH Biotin-11-dUTP

- ① Into a 1.5ml microcentrifuge tube add:
 

DNA template (100ng – 1µg)	10µl,
Decanucleotide in 5x Reaction Buffer	10µl,
Water, nuclease-free	to 44µl.

 Vortex the tube and spin down in a microcentrifuge for 3-5sec. Incubate the tube in a boiling water bath for 5-10min and cool it on ice. Spin down quickly.
- ② Add:
 

Biotin Labeling Mix	5µl,
Klenow fragment, $exo^-$ (5u)	1µl.

 Shake the tube and spin down in a microcentrifuge for 3-5sec. Incubate for 1 hour at 37°C. Prolonged incubation at 37°C up to 20 hours increases the yield of labeled DNA.
- ③ Stop the reaction by the addition of 1µl 0.5M EDTA, pH 8.0.
- ④ The labeled DNA is used directly for hybridization or stored at -20°C. Removal of the unincorporated label is not necessary for most applications. If required, the unincorporated dNTP can be removed by chromatography on Sephadex® G-50 or by selective precipitation of DNA with ethanol in the presence of ammonium acetate [3].

### CONTROL LABELING REACTION

- ① Into a 1.5ml microcentrifuge tube add:
 

λ DNA/HindIII fragments (10ng/µl)	25µl,
Decanucleotide in 5x Reaction Buffer	10µl,
Water, nuclease-free	9µl.

 Vortex the tube and spin down in a microcentrifuge for 3-5sec. Incubate the tube in a boiling water bath for 5-10min and cool it on ice. Spin down quickly.
- ② Add:
 

Biotin Labeling Mix	5µl,
Klenow fragment, $exo^-$ (5u)	1µl.

 Shake the tube and spin down in a microcentrifuge for 3-5sec. Incubate for 1 hour at 37°C. Incubation at 37°C for up to 20 hours increases the yield of labeled DNA.
- ③ Stop the reaction by the addition of 1µl 0.5M EDTA, pH 8.0.

### QUALITY CONTROL

All components of the kit are tested in a control labeling reaction. Labeled DNA probe is used for a spot hybridization. 0.3-0.1pg of homologous DNA is detected after 16 hours color development with a streptavidin conjugated to alkaline phosphatase which catalyzes a color reaction with 4-nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate (NBT/BCIP).

Quality authorized by:



Laima Kazilioniene

**References**

1. Feinberg, A.P., Vogelstein, B., Biochem. 132, 6-13, 1983.
2. Feinberg, A.P., Vogelstein, B., Biochem. 137, 266-267, 1984.
3. Sambrook, J., Fritsch, E.F. and Maniatis, T., Molecular Cloning: A Laboratory Manual; Second Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y., 1989.

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This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

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