Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific Biotin DecaLabel DNA Labeling Kit

#__ for __ reactions

Lot: __ Expiry Date: __

Store at -20°C

www.thermoscientific.com/onebio

COMPONENTS OF THE KIT

Component	#K0651 10 rxns	#K0652 30 rxns
Klenow Fragment, exo⁻, **5 u/µl**	15 µl	30 µl
Decanucleotide in 5X Reaction Buffer	100 µl	300 µl
Biotin Labeling Mix 1 mM dGTP, 1 mM dATP, 1 mM dCTP, 0.65 mM dTTP, 0.35 mM Biotin-11-dUTP aqueous solution	50 µl	150 µl
Control Template λ DNA/HindIII fragments,10 ng/µl	50 µl	125 µl
Biotin-labeled DNA 50 µl of biotin-labeled DNA (λ DNA/HindIII), 5 ng/µl	50 µl	125 µl
Water, nuclease-free	1.25 ml	2x1.25 ml

CERTIFICATE OF ANALYSIS

All components of the kit were tested in a control labeling reaction. Labeled DNA probe was used for a spot hybridization. 0.3-0.1 pg of homologous DNA was detected after 16 hours color development with a streptavidin conjugated to alkaline phosphatase which catalyzes a color reaction with NBT/BCIP.

Quality authorized by: Jurgita Zilinskiene

DESCRIPTION

Thermo Scientific Biotin DecaLabel DNA Labeling Kit is an advanced system for the efficient synthesis of biotin-labeled DNA probes, based on an improved random-primed labeling method originally developed by Feinberg and Vogelstein (1, 2). The primary improvement over the traditional random-primed method involves the use of random decamers instead of hexamers to ensure more efficient annealing with DNA at 37°C. Klenow Fragment, exo⁻ is also included in the kit; this genetically engineered enzyme has no detectable exonuclease activity. Therefore, the enzyme does not degrade the labeled probe during reaction, which results in a high labeling yield even with low amounts of template. As a result, DNA fragments of any length can be uniformly labeled. Biotin-labeled DNA is detected with the Biotin Chromogenic Detection Kit (#K0661) or with conventional biotin-avidin or biotin-streptavidin detection systems.

DNA LABELING with BIOTIN-11-dUTP

1. Add the following components into 1.5 ml microcentrifuge tube:

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

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

2. Add the following components in the same tube:

Biotin Labeling Mix	5 µl
Klenow fragment, exo⁻ (5 u)	1 µl

Shake the tube and spin down in a microcentrifuge for 3-5 s. 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 1 µl of 0.5 M 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. Add the following components into 1.5 ml microcentrifuge tube:

Control Template, 10 ng/µl	25 µl
Decanucleotide in 5X Reaction Buffer	10 µl
Water, nuclease-free	9 µl

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

2. Add the following components in the same tube:

Biotin Labeling Mix	5 µl
Klenow fragment, exo⁻ (5 u)	1 µl

Shake the tube and spin down in a microcentrifuge for 3-5 s. 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 1 µl of 0.5 M EDTA, pH 8.0.

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 Horbor laboratory, Cold Spring Harbor, N. Y., 1989.