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LABEL-IT™ 3' Biotin End Labeling Kit
Instruction Manual

Product:	LABEL-IT™ 3' Biotin End Labeling Kit (25 Reactions)
Product No:	LBL0325
Procedure:	Labeling DNA with biotin-14-dCTP (15.3)
Kit Storage:	Remove TRANSFER-IT™ Plus Membrane and store at RT. Store Kit at -20°C (non-frost free freezer is recommended).
Stability:	Performance of this kit is guaranteed for 6 months from date of purchase

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The LABEL-IT ™ 3' Biotin End Labeling Kit labels deoxyoligonucleotides with biotin at the 3' terminus generating probes that are used in a variety of non-isotopic detection methodologies. The enzyme utilized for this reaction, terminal deoxynucleotidyl transferase (TdT), is a DNA polymerase that catalyzes the addition of dNTPs (including biotin-14-dCTP) onto the 3' hydroxyl terminus of DNA. One to four biotin-14-dCTPs (average of 2.5) are added to the 3' terminus of either single stranded or double stranded DNA. The biotinylated probes are suitable for use in non-isotopic Northern blots, Southern blots, dot blots, *in situ* hybridizations, colony hybridizations and other hybridization-based assays. The 3' biotin end labeled DNA can also be used in solid phase-based nucleic acid applications, using the biotin as an anchor for binding to streptavidin-solid supports.

The LABEL-IT™ 3' Biotin End Labeling Kit provides all the reagents and buffers needed to perform 25 biotin-labeling reactions. Greater then 95% of the DNA is labeled with mostly two or three biotin-14-dCTPs added to the 3' hydroxy terminus of the molecule. Labeling is complete in 60 minutes. In addition to the reagents needed to complete the labeling reactions, the kit also contains a non-isotopic detection assay to assess the amount of biotin incorporated into the DNA.

Kit Contains:

25 µl	TdT (5 U/µl)
100 µl	5X TdT Reaction Buffer
25 µl	Biotin-14-dCTP (0.25 nmole/µl)
1 ml	Nuclease-Free Water (for reaction mixture)
5 µl	Control Oligonucleotide (13 pmole/µl)
50 µl	Control Biotinylated Oligonucleotide (2 pmole/µl)
2 x (2.5 x 7.5 cm)	TRANSFER-IT™ Plus Membrane
100 µl	HRP Conjugate (0.25 µg/µl) (Horseradish Peroxidase-Streptavidin)
2 x 1.5 ml	Colorimetric Detection Reagent
10 ml	10X Wash Buffer

Materials: (not included with the kit):

- Oligonucleotide to be labeled
- Distilled or deionized water
- 1.5 ml Microcentrifuge Tubes (nuclease-free)
- 37°C Water Bath
- Micropipettor
- Micropipette Tips (nuclease-free)
- Microcentrifuge
- Plastic Wrap

Biotin End Labeling Reaction

1. Prepare the reaction mixture by combining the following in a nuclease-free microcentrifuge tube:
   Nuclease-Free Water (enough to bring final volume to 20 µl)
   40 pmole oligonucleotide or other DNA to be labeled
   4 µl 5X TdT buffer
   1 µl Biotin-14-dCTP

2. Cut a 0.5 cm x 2.5 cm strip of TRANSFER-IT™ Plus Membrane from the larger piece supplied with the kit. Snip off a corner of the membrane strip to establish the lower left corner. Spot 1 µl of the reaction mixture onto the membrane strip and let air dry. (see diagram in *Determination of Labeling Efficiency* Section below)

   NOTE: Handle the membrane with gloved hands and forceps only

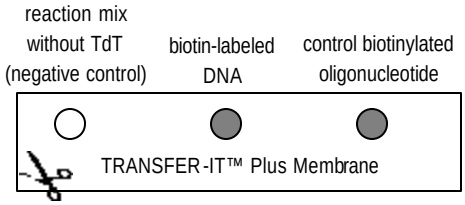
3. Add 1 µl of TdT to the reaction mixture. Mix the contents of the tube by gently flicking the bottom of the microcentrifuge tube. Centrifuge for 2 sec. (using a microcentrifuge) to bring all liquid to the bottom of the tube.

4. Incubate the tube for 1 hour at 37°C.

5. To stop the reaction heat for 5 minutes at 65°C. Spot 1 µl of the biotin-labeled DNA on the same strip of TRANSFER-IT™ Plus Membrane used in *Step #2.* Store the biotin-labeled DNA at -20°C.

6. *NOTE: The free Biotin-14-dCTP can be removed using spin-column chromatography or ethanol precipitation if needed.*

Determination of Labeling Efficiency



1. Spot 1.0 µl of Control Biotinylated Oligonucleotide on the same strip of membrane used in Steps 2 and 5 in the *Biotin Labeling Reaction* Section above (see diagram). Air dry the membrane strip at room temperature (10-15 minutes).

2. Pipette 125 µl of 10X Wash Buffer into 3 separate microcentrifuge tubes. Add 1125 µl distilled or deionized water to each tube and mix thoroughly.

3. Place the membrane into one of the microcentrifuge tubes prepared in Step 2. Incubate for 5 min. at room temperature, inverting the tube frequently.

4. Dilute the HRP Conjugate by adding 4 µl of the HRP Conjugate and 96 µl distilled or deionized water into a fresh microcentrifuge tube. Mix well by gently flicking the bottom of the microcentrifuge tube.

5. Remove membrane strip from the tube, drain the excess liquid from the membrane by touching the top edge of the membrane against a paper towel . Place the membrane strip on plastic wrap. Pipette a thin layer of the diluted HRP Conjugate solution (100 µl) onto the membrane. Incubate for 5 minutes at room temperature.

6. Place the membrane into the second microcentrifuge tube prepared in Step 2. Incubate for 5 min. at room temperature, inverting the tube frequently.

7. Remove the membrane strip from the tube and drain the excess liquid from the membrane by touching the top edge of the membrane against a paper towel. Place the membrane strip on plastic wrap and pipette a thin layer (100 µl) of the Colorimetric Detection Reagent onto the surface of the membrane.

8. Incubate at room temperature for 2 minutes and then place the membrane into the last microcentrifuge tube prepared in Step 2. Incubate for 1 minute at room temperature, inverting the tube frequently.

9. Remove the membrane from the microcentrufuge tube and compare the color intensity of the spots. The reaction mix without the TdT (negative control) should not develop color (although a very weak signal may be noticeable). The intensity of the spots on the membrane from the biotin-labeled DNA and the Control Biotinylated Oligonucleotide should be similar.

TECHNICAL NOTES

Failure of a biotinylation may be caused by a non-reactive DNA substrate, either due to anomalies of the DNA or the presence of inhibitors. The substrate for terminal deoxynucleotide transferase is single stranded or double stranded DNA which is at least 4 bases in length and contains a free hydroxyl on the 3' end of the molecule. If the labeling reaction fails, use the Control Oligonucleotide provided to test the performance of the kit by following the Labeling Reaction and Detection Protocols above.

If the Control Oligonucleotide labels well, it indicates that there is a problem with the original DNA substrate. An ethanol precipitation, or desalting on a spin column may remedy this problem. If the Control Oligonucleotide does not label well, please call technical services.

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