VECTOR LABORATORIES

part of Maravai LifeSciences

USER GUIDE

PHOTOPROBE® Biotin Reagents

Cat. No.: SP-1000 and SP-1020

Storage: -20 °C

DESCRIPTION

The PHOTOPROBE Biotin reagents offer a rapid, direct method for biotinylating nucleic acids. The system can be used to label single- or double-stranded DNA, RNA, oligonucleotides or peptide nucleic acids (PNA), eliminating the need for multiple labeling kits. This labeling strategy provides a convenient, economical alternative to enzymatic labeling.

A kit labels up to 250 µg of DNA, RNA or oligonucleotides or is sufficient for up to 50 labeling reactions. Complete labeling is accomplished in less than an hour.

KIT COMPONENTS

Product

PHOTOPROBE Biotin (SP-1000) **or** PHOTOPROBE Long Arm Biotin (SP-1020)

Tris Buffer (500 mM Tris, pH 9.5)

sec-butanol

Precipitant*

Biotinylated DNA standard

* Precipitant contains purified glycogen as well as sodium and magnesium salts.

STORAGE

Kit components should be stored at -20 °C. Before use, reconstitute PHOTOPROBE Biotin Reagent in 500 µl distilled water and store the solution in the dark at -20 °C. Under these conditions, the solution is stable for up to 1 year.

ADDITIONAL REAGENTS REQUIRED (NOT SUPPLIED)

- 95% ethanol
- 70% ethanol
- TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA)
- Benchtop microcentrifuge
- Light source for light labeling: mercury vapor bulb (120V, 250W), handheld 365 nm U.V. lamp, or desktop halogen lamp (15V, 50W), **OR** heating block for thermal labeling

PHOTOPROBE LABELING REACTION:

Two forms of labeling reagents are available, PHOTOPROBE Biotin (SP-1000) and PHOTOPROBE (Long Arm) Biotin (SP-1020) which contains an additional 7-atom spacer in the linker arm. Labeling and detection protocols are equivalent for the two forms.

PROTOCOL

For *in situ* hybridization applications, please ***See Note A***.

1. Dissolve nucleic acid sample in water or TE for photocoupling; in TE for thermal coupling. Concentration should not exceed 0.5 µg/µl. To a known volume (10 to 40 µl) of sample, add an equal volume of PHOTOPROBE Biotin Reagent.

2. **Photocoupling:** place the reaction tube (cap open) into an ice bath. Irradiate sample with one of the following light sources:

- 30 minutes with a mercury vapor lamp ~160w positioned 10 cm above the tube,
- 60 minutes with a halogen lamp positioned 5 cm above the tube,
- 30 minutes with a handheld U.V. lamp positioned 2 cm above the tube.

OR

Thermal coupling: overlay the reaction mixture with mineral oil and heat at 95 °C for 30 minutes using a heating block.

3. After coupling, remove most of the mineral oil (if used), bring the volume of labeling reaction to 144 µl with distilled water and add 16 µl of the Tris Buffer provided. ***See Note B.***

4. Add 160 µl of sec-butanol to the nucleic acid solution, vortex vigorously, and centrifuge (1 minute at 1,000 x g (ca. 4,000 rpm)) to separate the phases. Discard the upper butanol phase. Repeat the butanol extraction. ***See Note C.***

5. To precipitate the biotin-labeled nucleic acid, add 2.5 µl Precipitant and 150 µl 95% ethanol. Mix thoroughly. For PNA samples, replace the ethanol with one reaction volume of isopropanol.

6. Pellet the precipitated nucleic acid by centrifugation at 10,000 x g (ca. 13,000 rpm) for 15 minutes. Remove the supernatant and wash the pellet with 70% ethanol (wash PNA sample with isopropanol). Centrifuge at 10,000 x g for 5 minutes. Remove the supernatant, dry the pellet for several minutes and resuspend the nucleic acid in water or TE.

7. Applications requiring extremely pure nucleic acids (e.g. in situ hybridization, cell transformation) may need additional purification. Samples can be further purified and concentrated using the DNA-binding silica resin systems available from several suppliers or by using spin column gel filtration as described in ***Note D***.

See reverse side for notes and additional information.

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NOTES

A. Optimal probe length for *in situ* hybridization is less than 400 bases. Longer probes may cause decreased sensitivity and increased background. PHOTOPROBE labeling does not cleave DNA. If the sample is larger than 400 bases it is recommended to optimize probe size before labeling.

B. Raising the pH of the PHOTOPROBE-nucleic acid solution facilitates the removal of unincorporated PHOTOPROBE Reagent during the butanol extraction.

C. The volume of the aqueous phase will be reduced to approximately 40 µl.

D. To purify the labeled sample with spin column gel filtration, use a commercially available spin column or prepare a spin column with Sephadex® G-25 or other gel filtration matrix with a similar separation range (1-3 ml column volume). Wash the column three times with TEN buffer (10 mM Tris, pH 8.0, 1 mM EDTA, 100 mM NaCl) by centrifuging the buffer through the column. 1-2 minutes at 1,000 x g (ca. 4,000 rpm) is generally appropriate. Do not exceed the manufacturer's recommendations. Load the sample (in TEN buffer) onto the column and allow to equilibrate for 5 minutes. Centrifuge the sample through the column into a clean tube.

ESTIMATING LABELING EFFICIENCY

Successful labeling can be confirmed by comparing the labeled nucleic acid sample to the Biotinylated DNA standard in a side-by-side dot blot:

1. Dilute both the labeled nucleic acid sample and the Biotinylated DNA standard to 1 µg/ml, 100 ng/ml, 10 ng/ml, and 1 ng/ml in TE.

2. Dot 1 µl of each dilution on nitrocellulose or nylon membrane. Crosslink the membrane according to the manufacturer's protocol.

3. Block the membrane in blocking solution (Casein Solution, SP-5020, Animal-Free Blocker®, SP-5030, or R.T.U. Animal-Free Blocker and Diluent, SP-5035 can be used).

4. Detect by incubating with alkaline phosphatase - streptavidin (SA-5100) or appropriate alkaline phosphatase antibody conjugate and an AP substrate such as BCIP/NBT (SK-5400) or DuoLuX® Chemiluminescent/ Fluorescent Substrate (SK-6605) according to the instructions provided with the substrate. Efficiency of labeling can be estimated by the comparison of the signals of the labeled sample and the control DNA standard.