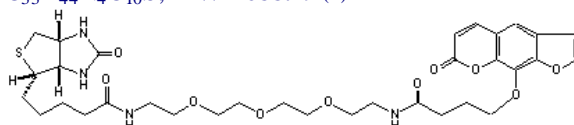


Psoralen-PEO4-Biotin

Product Description

Catalog number: UPL7784A, 10mg
 Name: **Psoralen-PEO4-Biotin**
 Formula : $C_{33}H_{44}N_4O_{10}S$; MW= **688.79** (L)



DNA/RNA probe modification does not interfere with hybridization 1
 Labeling occurs by photolysis at 350 nm, 10-30 min 2
 Psoralen moiety reacts through cycloaddition to the 5,6 double bond in thymine and other pyrimidine containing bases. 3
 Water soluble

Storage : +4°C, protected from light (L)

General Considerations

The biotin is a vitamin widely used in biotechnology for its propriety to bind with extremely high affinity to avidin ($K_a=10^{-15} M^{-1}$) and streptavidin ($K_a=10^{-14} M^{-1}$). This interaction hapten-protein resists effectively to drastic physico-chemical conditions, allowing various immuno-technologies. The biotin can be conjugated through several chemical reactions to molecules of interest, notably proteins, without modifying the biological activity of the molecule, thanks to its low molecular weight and steric volume. It is easily detected with labelled (strept)avidins, thus biotin represents a privileged label for antibodies and proteins involved in hapten-ligand interactions.

Interchim offers biotins activated by the psoralen group, that allows labeling of nucleotides upon UV illumination.

Directions for Use

This is a standard protocol for 500 µg of nucleic acid in a 10 µl reaction.
 A scale up and a setting may be needed to optimize the biotinylation level for each application.

Protocole 1 : biotinylation of an antibody

The reaction is advantagegely performed in 96-wells untreated microplates or any other suitable recipient as PCR microtubes (Axygen).
 For RNA, RNAases may be removed by soaking for 30min in 0.1M NaOH and rinsing.

- Prepare TE buffer:
10mM Tris HCl pH8.0, 1mM EDTA (can be stored at -20°C) with DNAase RNAse free distilled water.
- The nucleic acid solution should have a final concentration of 0.5-50 ng/µl (2mg/ml) in TE buffer. If not, the pH of the nucleic acid solution should be between 2.5 and 10, and the salt concentration should be less than 20 mM and EDTA be at 1mM.
- Double stranded DNA should be denatured:
Heat 500µg DNA in PCR microtubes for at least 10minutes at 100°C.
- Immediately quick-chill the sample:
Place the nucleic acid solution in a dry ice/alcohol bath, or liquid nitrogen, or ice water bath. Extremely rapid cooling is crucial for further efficient labelling. Leave the sample frozen until you are ready to add the psoralen.
- Dissolve 4.17µg of Psoralen-Biotin in 33µl dry DMF, protected from direct light. Store at +4°C and keep in the dark.
- Biotinylate:
Thaw the nucleic acid and add 1µl of Psoralen-Biotin solution to 10µl nucleic acid sample, and mix well, protected from light.
- Irradiate at 365nm for 45min:
Place the source(i.e. Philips TL 20W/09, 20nm bandwidth) directly on the microtubes (on on a microplate). Take care the UV source is less than 2 cm far from sample, and wavelenght is correct (. Plastic wrap or illumination from the bottom do not work properly. No degradation or crosslinking has been observed in these conditions, but do no irradiate over 1hour. As little as 15min may be sufficient.
- Purify the nucleic acid to remove excess of biotinylation reagent by a suitable technic: for example, .butanol extraction: add 200µl of water saturated n-Butanol (shake before use, use th top layer); vortex well, centrifuge 1 min at 7000g; wash with again 200µl. Redissolve th biotinylated nucleic acid in water or buffer for further use (hybridization)

Hybridizations

Psoralen biotinylated probes allow detection sensitivity of 100fg nucleic acid for 1 kb biotinylated DNA probe by blotting with chemiluminescent detection.

For hybridizations, here are some advice if you get too much background:

- .filter the probe and solution before use
- .Decrease the concentration, of the probe, and/or of the detector
- .Increase SD concentration in the hybridization and wash buffers up to 2% max.)
- .Increase the blocking time prior to and after incubation with the detector
- .Increase the wash time and/or number of washes after the blocking steps and detector step.

Scientific and Technical Information

- Psoralen-Biotin is water soluble which increases conjugation yields to water soluble biomolecules, and is easily removed by washing or dialysis . It is light sensitive
- Psoralen moiety is a planar tricyclic compound that has a natural affinity for nucleic acids and intercalates between bases as ethidium bromide does. It works with DNA, RNA, PCR products, cDNA insets and oligonucleotides. It overcomes classic phenyl azides. Intercalating in double stranded is better than in single stranded nucleotidic chains. 47-mers oligonucleotides have also been successfully been labelled. Crosslinking of the 2 strands of DNA may occur.
- It reacts under a brief irradiation of long wavelength (350/365 nm), 10-45 min through cycloaddition to the 5,6 double bond in thymine and other pyrimidine containing bases. It generates a covalently bonded biotin.
- Psoralen labeling should be done after denaturation of nucleic acids. Psoralen is not affected in presence of agarose; so a fragment of gel can be melted and labeled directly. Since psoralen does not react with water, it labelling can be done in aqueous solutions as organic co-solvents (DMSO, DMF, Ethanol...)
- DNA/RNA probe modification do not compromise hybridization efficiency, and are not subject to degradation making is ideal to create biotinylated nucleic probes.
- The polyethylene oxide (PEO) link confers superior water solubility, and extended spacer of 36.9 Angstroms length. In comparison with other classic biotinylation agents, it allows higher labeling concentrations, incomparable binding to (strept)avidin reagents, excellent stability of the conjugate, and finally increases the sensibility of detection.
- Psoralen biotinylated probes are stable at pH 2.5 to 10, and can be stored for long periods of time, for at least 1 year when stored frozen (best -80°C), alleviating the time consuming resynthesis of probes. The salt concentration must be kept below 20µM
- The level of biotinylation can be estimated by assay of biotin (dosage with HABA UP05361).

Other Information

Related products and documents:

PLP.Biotins

UPR0756

AMCH-Biotin

Literature:

1.Henriksen, U., et.al. (1991) Photochem. Photobiol. A: Chem. 57, 331-342.

2.Wassarman, D.A. (1993) Molec. Biol. Reports 17, 143-151.

3.Oser, A., et.al. (1988) Nucleic Acids Res. 16, 1181-1196.

4.Parsons B.J. (1980) Psoralen Photochemistry

For use in vitro only, not for diagnostic.

MSDS datas

Eye Protection: Use general lab eye protection-goggles.

Respiratory Protection: Not expected to require personal respirator. (Use NIOSH approved respirator as needed.)

Ventilation: Lab Hood as appropriate

Protective Gloves: Neoprene or PVC

Toxic. Harmful by inhalation. Avoid contact with skin, eyes and clothing.

Handling and Storage Considerations Laboratory aprons and gloves

Rev. D02E

Contact your local distributor

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