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SIGMA®

ProductInformation

PSORALEN AMINE
PSORALEN AMINE LABELING KIT

Product Codes **P 6100 and PS-AMINE**
Store at 2–8 °C
Technical Bulletin No. MB-650

TECHNICAL BULLETIN

Product Description

Psoralens, naturally occurring furocoumarins, have been used to study the structure and function of nucleic acids. These planar, tricyclic compounds intercalate into nucleic acids and upon irradiation with light between 320 to 400 nm, undergo a photocyclic addition, which covalently links the psoralen with the nucleic acid. Although photoaddition occurs primarily at thymidine and uridine in DNA and RNA respectively, minor reactivity with other bases has been reported. These molecules can be used to label single or double stranded nucleic acids.

Chemical labeling of nucleic acids with psoralens produces stable hybridization probes. The psoralen derivative contained here introduces a primary amine functional group to the nucleic acid, which can subsequently be used to tether a variety of molecules using standard amine conjugative chemistries. An advantage this has over traditional enzymatic labeling is the versatility of the reaction conditions and reproducibility of label incorporation. Preparation of amine containing probes by enzymatic incorporation (e.g. random prime or nick translation) is generally sensitive to conditions such as salt concentration, pH, nucleotide stability and concentration, template concentration, enzyme specific activity, and trace contaminants. By contrast, psoralen labeling can be performed over a wide range of pH (2.5 to 10.0) and template concentrations (0.5 to 50 µg/ml). However, salt concentrations in excess of 20 mM will inhibit psoralen intercalation and thus photoaddition. An obvious limitation of this labeling method is that the sequence must contain thymidines or uridines. The degree of psoralen addition ultimately depends upon the number of these bases and their proximities.

Precautions and Disclaimer

Crosslinking psoralen with nucleic acids requires use of UV irradiation which is hazardous to eyes and skin. Wearing proper protective equipment when using UV lights is strongly recommended. Sigma's Psoralen Amine Labeling Kit is for laboratory use only. Not for drug, household, or other uses.

Storage/Stability

Store the SigmaSpin™ Post-Reaction Purification Columns at 2–8 °C. For long term storage, store the control DNA and the psoralen amine reagent at –20 °C protected from light. The psoralen amine reagent is stable at –20 °C for at least one year.

Reagents Provided

The Psoralen Amine Labeling Kit contains reagents sufficient for 10 reactions, each with up to 2.5 µg of DNA

- Psoralen Amine, Product Code P 6100 12.5 µg per vial — 1 vial
- SigmaSpin™ Post-Reaction Purification Columns, Product Code S 0185 — 20 each
- Microcentrifuge Tubes, 2 ml Product Code T 7813 — 50 each
- Lambda DNA *Hin*d III Digest, Product Code D 9780 (Control DNA, 20 µg) — 1 vial
- N,N'-Dimethylformamide (DMF), Product Code D 4551 — 0.25 ml
- 100x Tris-EDTA (TE) Buffer, Product Code T 9285 — 0.5 ml

Materials Required but Not Provided

- Long wave UV source (365 nm, 8 W bulb)
- DNA or RNA to be labeled
- FITC, Product Code F 4274 or Biotin NHS ester, Product Code H 1759
- Nylon or nitrocellulose membrane
- 10x Blocking buffer, Product Code B 6429
- Tris buffered saline with TWEEN 20, Product Code T 9039
- Streptavidin-alkaline phosphatase, Product Code S 2890
- Anti-FITC-alkaline phosphatase antibody conjugate, Product Code A 5719
- BCIP/NBT liquid substrate, Product Code B 1911
- CDP-Star™, Product Code C 0712
- Autoradiographic film, Product Code F 1650
- Molecular biology grade water, Product Code W 4502

Preparation/General Instructions

1. Psoralen Amine
   a. Briefly centrifuge the tube containing the psoralen amine to collect all the solid on the bottom of the tube.
   b. Add 25 μl of DMF to the tube and heat at 65 °C for 3 minutes.
   c. Gently vortex to thoroughly dissolve the psoralen amine.
   d. Store at –20 °C between uses.

2. 1x TE Buffer
   Add 1 volume of 100x TE to 99 volumes of molecular biology grade water as needed (e.g. 10 μl of 100x TE buffer to 990 μl water).

3. SigmaSpin™ Post-Reaction Purification Column chromatography. Prepare just prior to use.
   a. Resuspend the resin by gently vortexing the column.
   b. Loosen the cap one-quarter turn and snap off the bottom closure.
   c. Place the column in an empty microcentrifuge tube.
   d. Centrifuge at 750 x *g* (3000 rpm for a microcentrifuge) for 1 minute. Dispose of the eluant.
   e. Remove the cap from the column, equilibrate the column by adding 400 μl of desired buffer to the column, replace the cap, and then centrifuge at 750 x *g* for 1 minute. Repeat equilibration one time. See the Protocol Section B, "Labeling Protocols", for suggested labeling reaction buffers.
   f. Place the equilibrated spin column into a fresh 1.5 ml microcentrifuge tube. Without touching the column sides or disturbing the resin bed add up to 50 μl of nucleic acid solution.
   g. Collect the purified nucleic acid in the microcentrifuge tube by centrifugation at 750 x *g* for 2 minutes.
   h. Return to crosslinking/conjugation procedure.

4. Control DNA
   a. Prepare 48 μl of control DNA at 50 μg/ml in 1x TE buffer.
   b. Heat denature at 100 °C for 10 minutes. Immediately place on ice for a minimum of 5 minutes. Psoralen crosslink and conjugate as described in the Procedure.
5. DNA for Labeling
   a. Dilute the DNA to 0.5 to 50 μg/ml in 48 μl of 1x TE buffer. Higher concentrations are recommended to ensure success.
   b. Heat denature the DNA at 100 °C for 10 minutes. Immediately place on ice for at least 5 minutes.
   c. Proceed to Nucleic Acid Psoralen Amine crosslinking procedure.

   Notes:
   - Any amount of DNA from 25 ng to 2.5 μg may be labeled.
   - Though scalable, it is recommended that multiple reactions be performed when starting with greater than 2.5 μg of DNA.
   - The amount of DNA to be labeled should be adjusted so that a sufficient amount of probe is produced. A minimum of 10 to 30 ng/ml of probe is typically required for hybridization experiments. The volume of probe solution required is dependent upon hybridization technique.
   - Heat denaturation prior to psoralen labeling is critical to avoid probe-target hybridization inhibition due to interstrand crosslinking.
   - The DNA should be in a low salt buffer prior to dilution into 1x TE buffer. Psoralen intercalation is inhibited by >20 mM salt. The DNA can be desalted by standard methods such as ethanol precipitation or size exclusion chromatography (e.g. SigmaSpin™ Post-Reaction Purification Columns).

6. RNA for Labeling
   a. Prepare an RNA sample in 1x TE buffer free of DNA, protein, salt, phenol, and ethanol. Assess purity by gel electrophoresis.

b. Measure the RNA concentration and adjust the concentration to 50 μg/ml.
c. Heat denature the RNA sample at 65 °C for 15 minutes.
d. Place on ice for a minimum of 5 minutes.
e. Proceed to Procedure: Nucleic Acid Psoralen Amine crosslinking.

Procedure

A. Nucleic acid Psoralen Amine crosslinking

1. Add 2 μl of psoralen amine solution to 48 μl of the nucleic acid solution. Mix and if necessary, collect the solution at the tube bottom by brief centrifugation.

2. Crosslink the psoralen amine to the nucleic acid with approximately 3 mJoules of 365 nm UV light. If using an 8 W 365 nm hand-held light source, expose the solution for 45 minutes on ice under dim light conditions with the light source positioned no less than 4 cm (1.5 in.) above the top of the solution. Empirical exposure optimization may be necessary if an alternative light source or crosslinking-oven is used.

   Notes:
   - Samples should not be irradiated from below the tube (e.g. with a transilluminator) as the plastic of the microcentrifuge tube will not fully transmit the UV light.
   - Short (254 nm) or medium wavelength (312 nm) illuminators should not be used for this procedure.
   - Though significant labeling occurs within 15 minutes of irradiation, we recommend a minimum of 45 minutes and a maximum of one hour exposure.

3. Remove excess psoralen amine using the SigmaSpin™ Post-Reaction Purification Column chromatography procedure (see Preparation/General Instructions above) using an appropriate equilibration buffer (i.e. the buffer that will be used in a conjugation step).

B. Conjugation/Labeling Protocols

1. Conjugation Buffers

   The recommended buffer for conjugating aminated DNA with NHS esters is 0.1 M NaH_2PO_4, pH 7.5., and for isothiocyanates 0.1 M $NaHCO_3$, 1 mM EDTA, pH 9.5, is recommended. Conjugation conditions for molecules other than biotin NHS ester and FITC may need specific optimization. The below examples may provide suitable starting points. The DMF concentration should be less than 5% in any procedure. Higher concentrations will adversely affect the performance of the size exclusion column.

2. Biotin/FITC Labeling

   a. Prepare a 10 mg/ml solution of N-hydroxy-succinimidobiotin (Product Code H 1759) or fluorescein isothiocyanate (Product Code F 4274) in DMF. Add 2 μl of the label to 48 μl of the psoralen amine crosslinked nucleic acid in the appropriate buffer (biotin NHS ester: 0.1 M NaH_2PO_4, pH 7.5, or FITC: 0.1 M $NaHCO_3$, 1 mM EDTA, pH 9.5)
   b. Gently agitate the solution for 60 minutes at room temperature.
   c. Remove excess label using the SigmaSpin™ Post-Reaction Purification Column size exclusion chromatography procedure equilibrated with 1x TE (see Preparation/General Instructions).
   d. The labeled nucleic acid may now be used as a probe in a variety of blotting techniques. A concentration of 10-30 ng/ml is recommended for initial hybridization experiments. Concentration optimization may be required.
   e. If the nucleic acid is too dilute, the sample may be lyophilized and reconstituted with molecular biology grade water to the required concentration.
   f. The nucleic acid probe is stable for up to one year when stored at –20 °C. Multiple freeze thaw cycles are **not** recommended. Large quantities of probe should be aliquoted and stored at –80 °C.

C. Validation of Label Incorporation

1. Spot 1 μl of 10x serially diluted probe, within the range of 50 ng/μl to 0.005 pg/μl, (control and user-labeled) onto neutral or positively charged nylon membrane. Labeled control DNA should be in approximately 50 μl of TE. If so, the concentration will be approximately 50 ng/μl.

2. Dry the membrane in an 80 °C oven for 3 to 5 minutes.

3. Crosslink the DNA to the membrane by exposure to UV light (120 mjoules).

4. Proceed with washing, antibody/streptavidin conjugate binding, detection etc. as per the CDP–*Star*™ Universal Detection Kit (Product Code U–ALK) technical bulletin.

5. The lower limit of detection with this method should be 0.05 to 50 pg.

D. Sample Chemiluminescent Hybridization Procedure

1. Dilute DNA template in denaturation solution (0.5 M NaOH with 1.5 M NaCl).

2. Denature probe by heating at 100 °C for 10 minutes. Immediately place on ice.

3. Spot 1.0 μl of DNA template on a membrane (nylon or nitrocellulose).

4. UV irradiate (130 mJoules) spotted membrane.

5. Wash blot in 2x SSC for 5 minutes to remove denaturation solution.

6. Pre-incubate the membrane in PerfectHyb™ Plus Buffer (Product Code H 7033) at 65 °C for 15 minutes.

7. Add denatured probe at a final concentration of 1:100 to 1:10,000 in pre-heated PerfectHyb Plus Buffer (Step 6). Hybridize at 65 °C for 4 hours to overnight.

8. Wash in 2x SSC to remove the majority of the unbound probe.

9. Stringency wash the blot twice for 15 minutes each with pre-heated (65 °C) 0.5x SSC with 0.1% SDS at 65 °C.

10. Wash briefly with 1x Wash Buffer (Product Code B 6564) to remove SDS from the stringency wash.

11. Proceed with washing, antibody/streptavidin conjugate binding, detection etc. as per the CDP–*Star*™ Universal Detection Kit (Product Code U–ALK) technical bulletin.

Troubleshooting Guide

Problem	Cause	Solution
Inefficient labeling	Inhibition of labeling reaction	If the reaction of the primary amine with the reactive label (i.e. NHS ester or isothiocyanate) does not occur, it is probably due to the presence of a contaminating free amine or wrong buffer. Ensure the DNA sample is in the proper buffer. For NHS esters use 0.1 M NaH_2PO_4, pH 7.5, and for isothiocyanates use 0.1 M $NaHCO_3$, 1 mM EDTA, pH 9.5, on the spin column.
	Inhibition of psoralen amine crosslinking due to high salt	In the presence of ≥20 mM salt, crosslinking will not occur. Ethanol precipitation or gel filtration may be used to desalt the nucleic acid solution prior to the crosslinking reaction.
	DNA concentration is too high or psoralen amine concentration is too low.	If the ratio of psoralen amine to nucleic acid becomes low, the nucleic acid will contain a low level of photoaddition product. Verify nucleic acid concentration and dilute the sample to be within the recommended range prior to psoralen crosslinking.
	Nucleic acid degraded	Ensure sample integrity prior to crosslinking reaction.
	Inadequate exposure to UV light	Increase the time of UV irradiation by 10 minute increments.
	Incorrect UV wavelength	Verify that UV light source is at 365 nm.

Reference

Cimino, *et. al.,* Ann. Rev. Biochem., **54,**1151–1193 (1985).

Related Products

Product Name	Product Code
PerfectHyb Plus	H 7033
Southern Breeze Blotting Kits	SBRZ-1A SBRZ-1B SBRZ-2A SBRZ-2B
Alkaline Southern Breeze Blotting Kits	ASBRZ-1A, ASBRZ-1B, ASBRZ-2A, ASBRZ-2B
Northern Breeze Blotting Kits	NBRZ-1A, NBRZ-1B, NBRZ-2A, NBRZ-2B
Alkaline Southern Transfer Solution	A 7967
Alkaline Southern Transfer Neutralizing Solution	A 8092
Neutral Southern Transfer Solution	N 0907
Neutral Southern Transfer Denaturing Solution	N 1531
Neutral Southern Transfer Neutralizing Solution	N 1532
Neutral Southern Transfer Depurination Solution	N 1907
Neutral Northern Transfer Solution	N 6531
Mild Alkaline Northern Transfer Solution	A 8217
Mild Alkaline Northern Wash Solution	A 8342
BioBond™ and BioBond-Plus™ Nylon Membrane	

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