Sunnyvale Red™ SE, a Superior Replacement for 6-ROX SE

Introduction

6-ROX and its derivative are widely used for labeling oligonucleotides and automated DNA sequencing applications. Although ROX exhibit excellent spectral properties for labeling biological molecules, ROX acid and SE compounds are quite unstable. They degrade quite significantly during storage. Sunnyvale Red™ dyes have been developed to retain the spectral properties of ROX fluorophores while their stabilities are significantly enhanced.

Spectral Properties:

Maximum Excitation: 571 nm
Maximum Emission: 596 nm
Recommended Filter: Texas Red® Filter

Use of Sunnyvale Red™, SE for Labeling Amino-Modified Oligonucleotides

Stock Solvent

Sunnyvale Red™, SE reagents should be dissolved in high-quality dimethylsulfoxide (DMSO) before reaction with amine-modified oligonucleotides.

Reaction Buffer

Sunnyvale Red™, SE reagents will react with the non-protonated amine group on the modified oligonucleotide. In order to maintain this amine group in the non-protonated form, the conjugation must take place in a buffer with slightly basic pH. For optimal results we recommend using a tetraborate buffer at pH 8.5, rather than the bicarbonate buffers recommended for protein conjugations.
[Note: It is important to avoid buffers that contain primary amines, such as Tris, as these will compete for conjugation with the amine-reactive compound.]

Labeling Protocol

The protocol has been optimized for labeling 100 µg of an 5′-amine–modified oligonucleotide, 15 to 25 bases in length. Some adjustments to the protocol may be necessary for greatly shorter or longer oligonucleotides. The reaction may be scaled up or down as long as the concentration of each component is not changed.

a). Purify the amine-modified oligonucleotide (if necessary). Dissolve the oligonucleotide in 100 µL dH2O and extract three times with an equal volume of chloroform. Precipitate the oligonucleotide by adding one-tenth volume (10 µL) of 3 M NaCl and two and a half volumes (250 µL) of cold absolute ethanol. Mix well and place at ≤–20°C for 30 minutes. Centrifuge the solution in a microcentrifuge at ~12,000 g for 30 minutes. Carefully remove the supernatant, rinse the pellet once or twice with cold 70% ethanol and dry under vacuum. Dissolve the dry pellet in dH2O to achieve a final concentration of 25 µg/µL. This amine-modified oligonucleotide stock solution may be stored frozen at≤–20°C.
[Note: To ensure that the oligonucleotide is free of interfering compounds, especially amines (such as triethylamine or Tris) and ammonium salts, we strongly recommend extracting and precipitating the sample prior to initiating the labeling reaction.]

b). Prepare labeling buffer. Make a 0.1 M sodium tetraborate buffer by dissolving 0.038 g of sodium tetraborate decahydrate for every mL of water. Adjust pH with HCl to 8.5.
[Note: This labeling buffer should be made as close as possible to the time of labeling. Alternatively, it may be divided into small aliquots and frozen immediately for long-term storage. Exposure of this solution to air for a long time will result in carbon dioxide absorption, which will change the pH of the buffer.]

c). Prepare Sunnyvale Red™ SE DMSO Stock Solution. Allow Sunnyvale Red™ SE to come to room temperature before opening the vial. Dissolve 300 µg of Sunnyvale Red™ SE in 10 µL DMSO by pipetting up and down, washing the sides of the vial. Use 200 µg Sunnyvale Red™ SE for labeling 100 µg of oligonucleotide.
[Note: It is important that Sunnyvale Red™ SE be freshly prepared for each labeling reaction as it is not stable in solution.]

d). Start the conjugation. To the vial containing Sunnyvale Red™ SE labeling reagent in DMSO, add 7 µL dH2O, 75 µL labeling buffer (step b), 4 µL of a 25 µg/µL oligonucleotide stock solution (step a). Place the vial on a shaker oscillating at low speed or gently vortex mix or tap the vial every half hour for the first two hours to ensure that the reaction remains well mixed. Do not mix

violently, as material may be left on the sides of the vial. After six hours, 50–90% of the amine-modified oligonucleotide molecules should be labeled.
[Note: The reaction mixture may have a grainy appearance, but this should not adversely affect the conjugation. The reaction may be scaled up or down as long as the concentration of each component
is not changed. Do not add more dye than recommended, as excess dye will not improve the labeling efficiency and may make the purification more difficult.]

e). Purifying the Sunnyvale Red™-Labeled Oligonucleotide. Following the reaction, the labeling mixture contains labeled oligonucleotide, unlabeled oligonucleotide, and unincorporated dye. Precipitate the reaction mixture with ethanol as follows: Add one-tenth volume of 3 M NaCl and two and a half volumes of cold absolute ethanol to the reaction vial. Mix well and place at ≤–20°C for 30 minutes. Centrifuge the solution in a microcentrifuge at ~12,000 × g for a full 30 minutes. The labeled oligonucleotide can be further purified by preparative gel electrophoresis or reverse-phase HPLC.
[Note: Loss of sample may occur if the centrifugation is not long enough. Carefully remove the supernatant, rinse the pellet once or twice with cold 70% ethanol and dry briefly. If the labeled oligonucelotide becomes completely dry, it will be difficult to redissolve.]

f). HPLC Purification: Use a standard analytical (4.6 × 250 mm) C8 column. Dissolve the pellet from the ethanol precipitation in 0.1 M TEAA (triethylammonium acetate). Load the dissolved pellet onto the column in 0.1 M TEAA and run a linear 5–65% acetonitrile gradient over 30 minutes. This gradient is a 2% increase in acetonitrile per minute. The unlabeled oligonucleotide will migrate fastest, followed by the labeled oligonucleotide and finally the free dye.

g). Gel Eelectrophoresis Purification: To purify the labeled oligonucleotide by gel electrophoresis, pour a 0.5 mm–thick polyacrylamide slab gel. For oligonucleotides less than 25 bases in length, use 19% acrylamide, for oligonucleotides 25–40 bases, 15% acrylamide, and for oligonucleotides 40–100 bases, 12% acrylamide. Resuspend the pellet from ethanol precipitation in 200 µL of 50% formamide, and incubate at 55°C for 5 minutes to disrupt any secondary structure. Load the warmed oligonucleotide onto the gel (you may need to use several wells) and load an adjacent well with 50% formamide plus 0.05% bromophenol blue. The bromophenol blue will migrate at approximately the same rate as the oligonucleotide. Run the gel until the bromophenol blue indicator dye is two-thirds of the way down the gel. Remove the gel from the glass plates and place on Saran Wrap. Lay the gel on a fluorescent TLC plate. Locate the labeled and unlabeled oligonucleotides by illumination with a handheld UV source. Fluorophore-labeled oligonucleotides will show fluorescence when illuminated with UV light. Cut out the band containing the labeled oligonucleotide and purify by the "crush-and-soak" method or other suitable method.

Other Biological Applications:

The Sunnyvale Red™ fluorophore emits at a longer wavelength than do either tetramethylrhodamine or Lissamine rhodamine making Sunnyvale Red™ conjugates among the most commonly used long-wavelength "third labels" in fluorescence microscopy. Unlike the other rhodamines, the Sunnyvale Red™ fluorophore exhibits very little spectral overlap with fluorescein, and its fluorescence can be distinguished from that of phycoerythrins. Moreover, the fluorescence quantum yield of Sunnyvale Red™ conjugates is usually high. When the correct optical filter sets are used, Sunnyvale Red™ conjugates are brighter and have lower background than conjugates of the other commonly used red-fluorescent dyes. Sunnyvale Red™ conjugates are particularly well suited for excitation by the 568 nm spectral line of the Ar–Kr mixed-gas laser now used in many confocal laser-scanning microscopes, or the 594 nm spectral line of the orange He–Ne laser.

Storage Conditions:

Store container at -20 °C. Expiration date is one year from the date of receipt.
[Note: Sunnyvale Red is moisture-sensitive. Store the reagent vial in desiccant. Equilibrate vial to room temperature before opening to avoid moisture condensation inside the container. Dissolve needed amount of reagent and use it immediately before hydrolysis occurs. Discard any unused reconstituted reagent. Do not store reagent in solution.]

References:

1. Oliver R.W.A., *HPLC of Macromolecules: A Practical Approach*, IRL Press (1989).
2. Sambrook J., Fritsch E.F., and Maniatis, T., *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring Harbor Laboratory (1989).