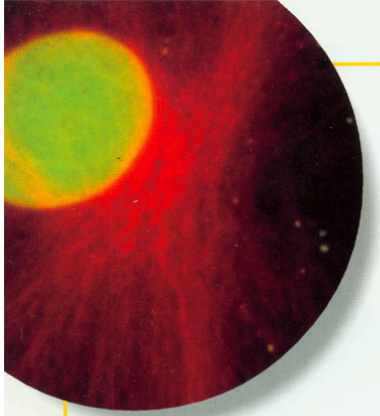

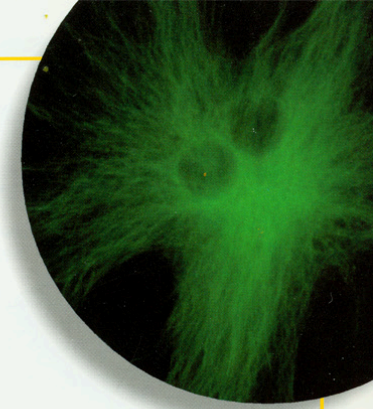

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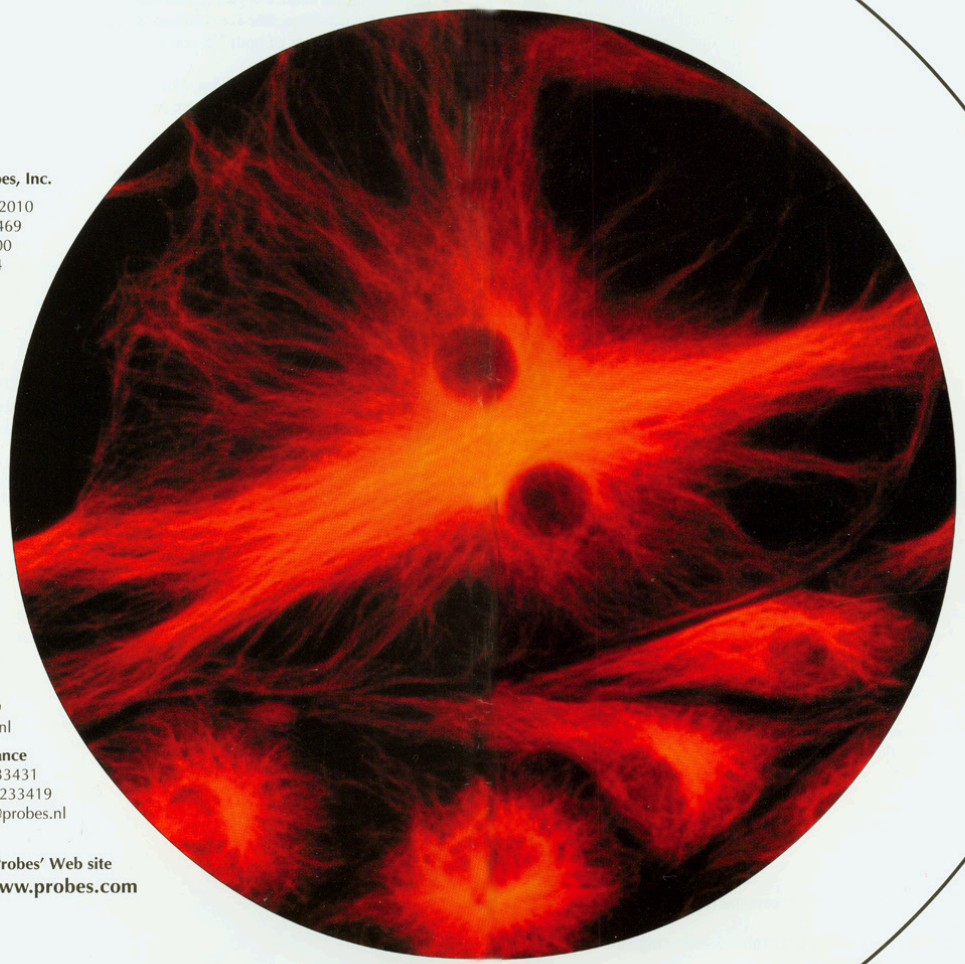
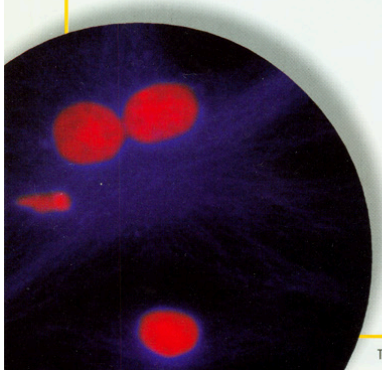
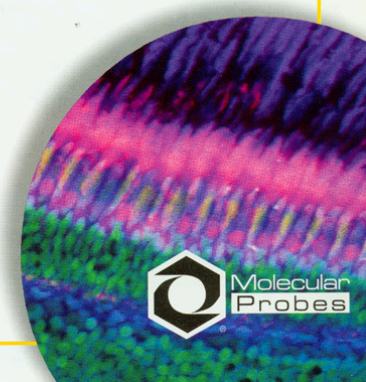


Superior Dyes for Protein Conjugation

Molecular Probes

The Leader in Fluorescence Technology





TC0143

Red-Orange

Rhodamine Red-X Succinimidyl Ester — A Superior Alternative to Lissamine Rhodamine B Sulfonyl Chloride

Absorption/Emission ~ 570/590 nm

Rhodamine Red™-X succinimidyl ester provides the benefits of the Lissamine™ rhodamine B fluorophore, including good spectral separation from green fluorophores and compatibility with 568-nm excitation sources, with all of the advantages of the succinimidyl ester chemistry.

Rhodamine Red-X goat anti–mouse IgG

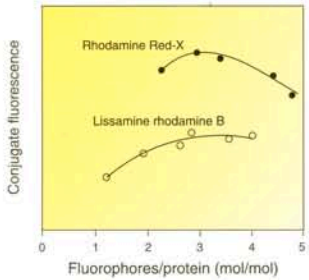


Figure 4. Rhodamine Red-X succinimidyl ester yields higher fluorescence output per attached dye than does Lissamine rhodamine B sulfonyl chloride.[4] The relative fluorescence of goat anti–mouse IgG conjugates prepared with Rhodamine Red-X succinimidyl ester and Lissamine rhodamine B sulfonyl chloride is plotted versus the number of fluorophores attached per protein.

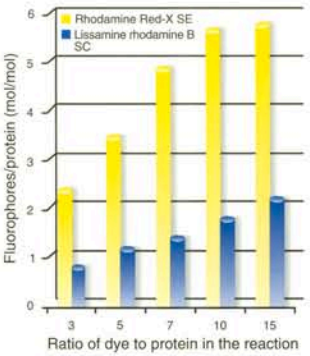


Figure 5. Rhodamine Red-X succinimidyl ester (SE) consistently yields a greater labeling efficiency than Lissamine rhodamine B sulfonyl chloride (SC).[4] Reactions with the same ratio of dye to protein were performed using streptavidin as the protein. After purification to remove free dye, the number of fluorophores per protein was determined for each conjugate. Conjugations with the SE were performed at room temperature and pH 8.3. Conjugations with the SC were performed at 4°C at pH 9.

Benefits of Rhodamine Red-X Succinimidyl Ester

- **Brighter conjugates** — less quenching upon conjugation produces conjugates that are more fluorescent (Figure 4)
- **Easier conjugations** — much slower hydrolysis rate, so less dye is required for conjugation (Figure 5)
- **Less batch-to-batch variability** — succinimidyl esters are much more stable than sulfonyl chlorides, which are extremely sensitive to even trace amounts of moisture
- **Less precipitation of conjugates** — less loss due to protein precipitation results in higher reaction yields

Protein conjugates prepared with Lissamine rhodamine B have fluorescence emission spectra that lie between those of tetramethylrhodamine and Texas Red® dyes and are maximally excited by the 568-nm line of the Kr-Ar mixed-gas laser used in many confocal laser scanning microscopes. Unfortunately, Lissamine rhodamine B is a sulfonyl chloride, which is highly unstable in aqueous solution. To overcome this limitation, Molecular Probes modified Lissamine rhodamine B by adding an aminohexanoyl spacer between the fluorophore and the reactive group and changing the reactive group to a succinimidyl ester. The result is our proprietary Rhodamine Red-X dye. With higher reaction yields and a slower hydrolysis rate, the succinimidyl ester of the Rhodamine Red-X dye represents a major improvement over Lissamine rhodamine B sulfonyl chloride.

		Unit Size
R-6160	Rhodamine Red™-X, succinimidyl ester *mixed isomers*	5 mg

Red

Texas Red-X Succinimidyl Ester — A Major Improvement over Texas Red Sulfonyl Chloride

Absorption/Emission ~ 595/615 nm

Molecular Probes' proprietary Texas Red®-X succinimidyl ester provides significant advantages over Texas Red sulfonyl chloride, including easier conjugation and greater labeling efficiency.[4] In fact, we have been so pleased with the performance of the Texas Red-X succinimidyl ester that we use it to prepare nearly all of our Texas Red conjugates.

Texas Red-X goat anti–mouse IgG and SYTOX® Green nucleic acid stain

Benefits of Texas Red-X Succinimidyl Ester

- **Easier conjugations** — much slower hydrolysis rate than Texas Red sulfonyl chloride, so ~ 50% less dye is required to achieve the same fluorophore-to-protein ratio (Figure 6)
- **Brighter conjugates** — less quenching upon conjugation produces conjugates that are more fluorescent (Figure 7)
- **Greater selectivity** — succinimidyl esters are much more amine-selective than sulfonyl chlorides, which can react to form unstable products with tyrosine, histidine and cysteine
- **Less batch-to-batch variability** — succinimidyl esters are much more stable than sulfonyl chlorides, which are extremely sensitive to even trace amounts of moisture
- **Less precipitation of conjugates** — less protein precipitation during the reaction and in storage means higher reaction yields and increased shelf lives

Texas Red conjugates are the most commonly used "third labels" in fluorescence microscopy and flow cytometry, primarily because the Texas Red fluorophore emits at a longer wavelength than either tetramethylrhodamine or Lissamine™ rhodamine B. Our Texas Red-X succinimidyl ester combines the strengths of the Texas Red fluorophore with the improved brightness, stability and reaction yields of the succinimidyl ester form. And because the succinimidyl ester has a slower hydrolysis rate, you need only about half as much of the reactive dye as you would of the Texas Red sulfonyl chloride to get the same fluorophore-to-protein ratio (Figure 6). We know you will be impressed with the easier and more reproducible conjugations that this dye provides.

		Unit Size
T-6134	Texas Red®-X, succinimidyl ester *mixed isomers*	5 mg

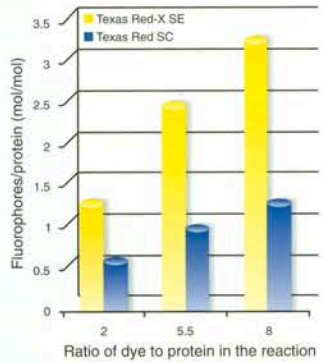


Figure 6. Texas Red-X succinimidyl ester (SE) consistently yields a greater labeling efficiency than Texas Red sulfonyl chloride (SC).[4] Reactions with the same ratio of dye to protein were performed using goat anti–mouse IgG as the protein. After purification to remove free dye, the number of fluorophores per protein was determined for each conjugate. Conjugations with the SE were performed at room temperature and pH 8.3. Conjugations with the SC were performed at 4°C and pH 9.

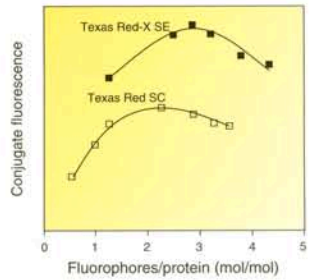


Figure 7. Texas Red-X succinimidyl ester (SE) yields conjugates with greater fluorescence than does Texas Red sulfonyl chloride (SC).[4] The relative fluorescence of goat anti–mouse IgG conjugates prepared with Texas Red-X SE and Texas Red SC is plotted versus the number of fluorophores attached per protein.