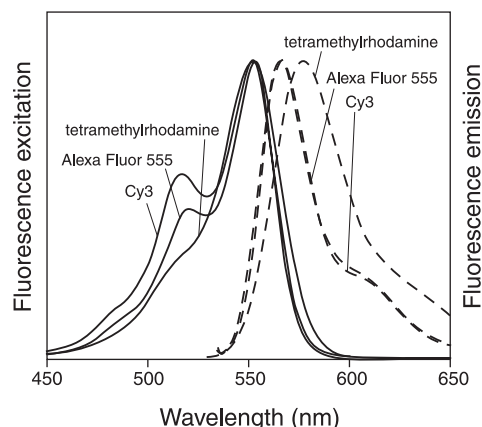


## Alexa Fluor® 555 Dye—A Superior Alternative to Tetramethylrhodamine and Cy3.

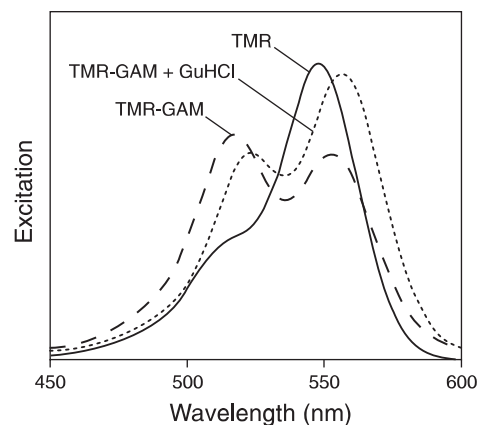
Tetramethylrhodamine is a time-honored red-orange fluorophore for protein labeling, yet it is not without its limitations. The Alexa Fluor®, 555 dye, which exhibits fluorescence excitation and emission spectra that are almost identical to those of tetramethylrhodamine and the Cy3 dye (Figure 1), overcomes several of these obstacles:

- **Increased photostability.** The red-orange–fluorescent Alexa Fluor® 555 dye is significantly more photostable than both tetramethylrhodamine and Cy3, allowing more time for image capture.
- **High extinction coefficient.** The extinction coefficient of the succinimidyl ester of Alexa Fluor® 555 carboxylic acid ( $155,000 \text{ cm}^{-1}\text{M}^{-1}$ ) is significantly larger than that of the succinimidyl ester or isothiocyanate derivatives of tetramethylrhodamine ( $92,000 \text{ cm}^{-1}\text{M}^{-1}$  and  $99,000 \text{ cm}^{-1}\text{M}^{-1}$ , respectively)
- **Brighter protein conjugates.** Alexa Fluor® 555 protein conjugates are appreciably brighter than both tetramethylrhodamine and Cy3 protein conjugates. This superior brightness is due in part to the observation that the absorption spectrum of tetramethylrhodamine-labeled proteins frequently splits into two absorption peaks, with the shorter-wavelength peak corresponding to a nonfluorescent dye aggregate (Figure 2). Protein conjugates of our Alexa Fluor® 555 dye show normal absorption spectra with correspondingly high extinction coefficients.

Since its introduction in 2002, the Alexa Fluor® 555 dye has been cited in close to 200 journal articles, many of which describe the direct substitution of the Alexa Fluor® 555 dye in experiments that formerly used either tetramethylrhodamine or Cy3. With a variety of reactive forms of the Alexa Fluor® 555 dye available, as well as a wide selection of protein conjugates (Table 1), we offer many choices for replacing tetramethylrhodamine in your research. And because the Alexa Fluor® 555 dye (excitation/emission maxima  $\sim 555/565 \text{ nm}$ ) is such an excellent spectral match to tetramethylrhodamine, you won't have to change the instrumentation or the other fluorescent parameters in your experiment.



**Figure 1.** Fluorescence excitation and emission spectra for Alexa Fluor® 555 dye, Cy3, and tetramethylrhodamine.



**Figure 2.** Effect of protein conjugation on the excitation spectrum of tetramethylrhodamine. The excitation spectrum of tetramethylrhodamine conjugated to goat anti-mouse IgG antibody (TMR-GAM) shows an additional peak at about 520 nm when compared with the spectrum of the same concentration of the free dye (TMR). Partial unfolding of the protein in the presence of 4.8 M guanidine hydrochloride (TMR-GAM + GuHCl) results in a spectrum more similar to that of the free dye.

**Table 1.** If you are using tetramethylrhodamine probes, consider switching to these Alexa Fluor® 555 probes for enhanced brightness and photostability.

Fluorophore Derivative	Cat # of TMR Derivative	Cat # of Alexa Fluor® 555 Derivative
Amine-Reactive Derivatives	T490, T668, C1171, T1480, T1481, C2211, T6105, C6123	A20009, A20109, A32755, A32756
Protein Labeling Kits	F6163	A20174, A20187, A30007, Z25005, Z25105, Z25205, Z25305, Z25405, Z25605
Thiol-Reactive Derivatives	T6006, T6027, T6028	A20346, H30463
Amine-Containing Derivatives	A1318	A30677
Avidin and Streptavidin Conjugates	S870, A6373	S21381, S32355, I37162
Secondary Antibodies—goat anti-mouse IgG conjugates	T2762	A21127, A21137, A21147, A21157, A21422, A21424, A21425, A21427, I37152
Secondary Antibodies—goat anti-rabbit IgG conjugates	T2769	A21428, A21429, A21430, I37157
Transferrin Conjugate	T2872	T35352
Epidermal Growth Factor Conjugate	E3481	E35350
Phalloidin	R415	A34055
Bovine Serum Albumin (BSA)	A23016	A34786
Wheat Germ Agglutinin (WGA)	W849	W32464
Dextran	D1816, D1817, D1868	D34679
α-Bungarotoxin	T1175	B35451

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