Technical Tip: Greater Control and More Reaction Time with SDP Ester

Activated esters, such as the *N*-hydroxysuccinimidyl (NHS) ester are the preferred method for attaching the bright, photostable Alexa Fluor® dyes to primary amines on molecules of interest, including antibodies and amine-modified oligos for use in imaging and flow cytometry applications as a result of the stable carboxyamide bond that they form[1] (Figure 1). Although an easy and efficient means for attaching fluorophores, activated esters are also subject to hydrolysis. Of all of the Alexa Fluor® dyes, the Alexa Fluor® 488 NHS ester is the most hydrolytically unstable. Typically, in order to achieve efficient labeling, one must compensate for the loss of the material to this unwanted side-reaction with an excess of material to achieve the desired result. The tetrafluorophenyl ester or TFP ester was developed as an improved alternative to the NHS ester. With its improved hydrolytic stability, researchers using the TFP ester (under similar reaction conditions as an NHS ester) can achieve higher degree of labeling (Figure 2). The TFP ester will still hydrolyze at elevated pH where most amine-conjugations reactions occur. Thus, the only way to control the degree-of-labeling (DOL) with either the TFP or NHS activated esters is to alter the amounts or molar ratio (MR) of the activated ester to the molecule of interest.

The novel sulphodichlrophenyl ester (SDP) provides researchers with the first, truly hydrolytically stable form of the Alexa Fluor® 488 dye for amine-conjugations (Figure 3). Now in addition to adjusting the MR, one can also use time to control the resulting DOL and often use less dye to achieve optimum results.

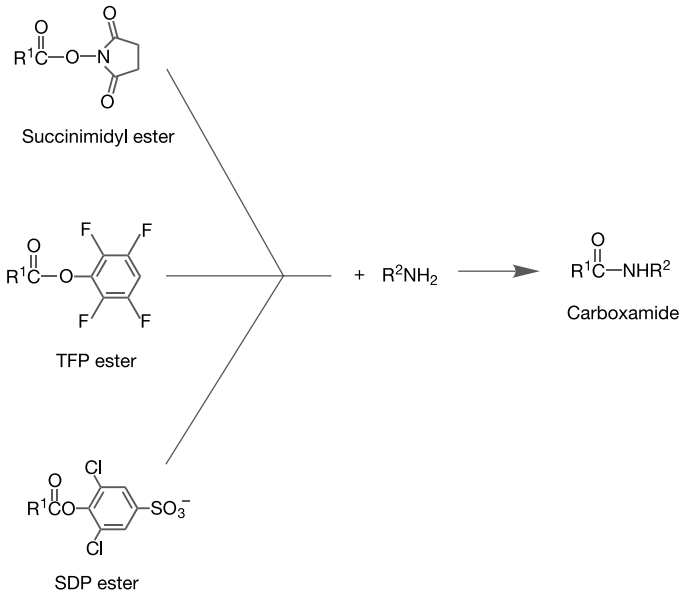

Figure 1. Reaction of a primary amine with a succinimidyl, a tetrafluorophenyl (TFP), or a sulfodichlorophenyl (SDP) ester.

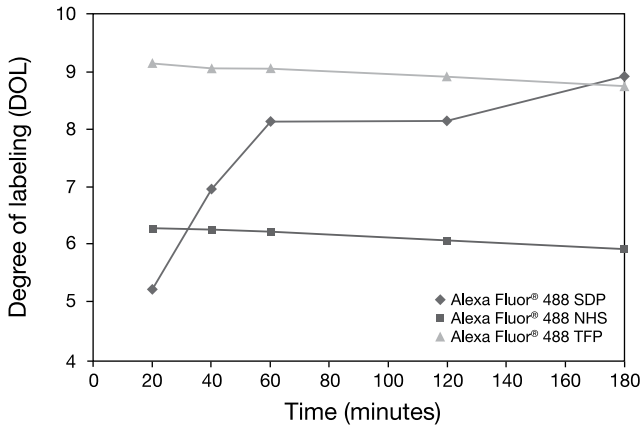

Figure 2. Comparison of the reaction kinetics for Alexa Fluor 488 sulfodichorophenyl (SDP) ester, tetrafluorophenyl (TFP) and *N*-hydroxysuccinimidyl esters with goat anti-mouse (GAM) IgG antibody under standard amine-conjugation reaction conditions. After normalizing the SDP, TFP or NHS ester to 100% reactivity, they were reacted with GAM IgG antibody at a molar ratio of 12 and the resulting degree of labeling (DOL) was measured at various time points.

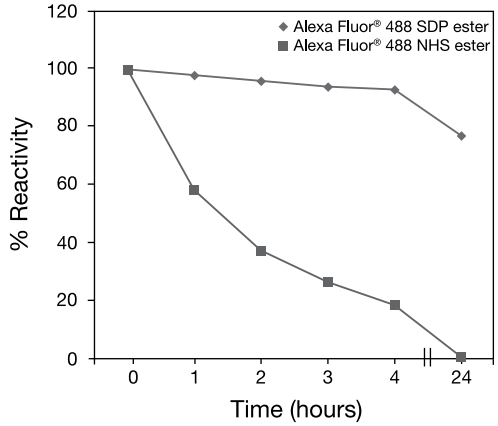

Figure 3. The sulfodichorophenyl (SDP) ester is significantly more hydrolytically stable than *N*-hydroxysuccinimidyl (NHS) esters under typical amine-conjugation reaction conditions. Equivalent amounts of either the SDP or NHS ester normalized to 100% reactivity were incubated in phosphate buffer at pH 8.6. At identified time points, the material was reacted with and excess of n-butylamine and analyzed by HPLC to determine remaining percent reactivity.

Reference

1. Comparison of Three Common Amine Reactive Fluorescent Probes Used for Conjugation to Biomolecules by Capillary Zone Electrophoresis." Banks PR, Paquette DM. Bioconjug Chem 6, 447-458 (1995)

Product List

Cat #	Product Name	Unit Size
A30052	Alexa Fluor® 488 5-SDP ester (Alexa Fluor® 488 sulfodichlorophenol ester)	1 mg

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