

## Alexa Fluor® Succinimidyl Esters

**Table 1.** Contents and storage information.

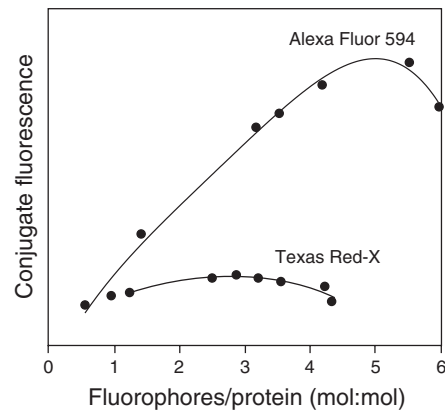
Material	Amount	Storage	Stability
Alexa Fluor® succinimidyl esters	100 µg	<ul style="list-style-type: none"> <li>• ≤-20°C</li> <li>• Desiccate</li> <li>• Protect from light</li> </ul>	When stored as directed, reactive dyes are stable for at least 3 months.
	1 mg		
	5 mg		

**Approximate fluorescence excitation and emission maxima:** See Table 4.

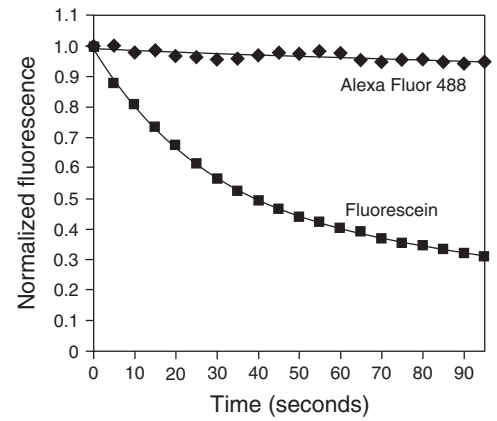
### Introduction

The Alexa Fluor® dyes are superior fluorophores with fluorescence emissions that span the visible spectrum and beyond. Alexa Fluor® conjugates exhibit brighter fluorescence (Figure 1) and greater photostability (Figure 2) than the conjugates of other spectrally similar fluorophores. These characteristics allow you to capture images that were previously unattainable with conventional fluorophores. The dyes in this series are also water soluble and pH insensitive from pH 4–10, to aid researchers who are working in biological environments.

The succinimidyl esters of the Alexa Fluor® dyes are available as standalone reagents (Table 2), giving you the freedom to create optimal Alexa Fluor® conjugates by developing custom labeling schemes. These succinimidyl esters provide an efficient and convenient way to selectively link the superior Alexa Fluor® dyes to primary amines (R-NH<sub>2</sub>) located on peptides, proteins, or amine-modified nucleic acids. Unlike other reactive moieties, succinimidyl esters demonstrate very low reactivity with aromatic amines, alcohols, and phenols, including tyrosine and histidine. Succinimidyl esters are preferred over other amine-reactive reagents, such as isothiocyanates, for attaching fluorophores to amine-containing molecules, because the amide bonds formed in the reaction are as stable as peptide bonds.<sup>1</sup>



**Figure 1.** Comparison of the relative fluorescence of goat anti-mouse F(ab)<sub>2</sub> fragments labeled with Alexa Fluor<sup>®</sup> 594 or Texas Red<sup>®</sup>-X dye at different dye:protein ratios.



**Figure 2.** Photobleaching profiles of cells stained with Alexa Fluor<sup>®</sup> 488 dye or fluorescein conjugates. HEP-2 cells were probed with human anti-human nuclear antibodies and then with mouse anti-human IgG. Finally, the cells were detected using F(ab)<sub>2</sub> fragments of goat anti-mouse IgG labeled with Alexa Fluor<sup>®</sup> 488 dye or fluorescein. Samples were continuously illuminated and images were collected every five seconds with a cooled CCD camera. Normalized data demonstrate the difference in photobleaching rates.

**Table 2.** Alexa Fluor<sup>®</sup> succinimidyl esters.

Alexa Fluor <sup>®</sup> Dye	Succinimidyl Ester	
	1 mg Unit Size	5 mg Unit Size
Alexa Fluor <sup>®</sup> 350	NA	A10168
Alexa Fluor <sup>®</sup> 405	A30000	A30100
Alexa Fluor <sup>®</sup> 430	NA	A10169
Alexa Fluor <sup>®</sup> 488	A20000	A20100
Alexa Fluor <sup>®</sup> 500	A30001	Inquire
Alexa Fluor <sup>®</sup> 514	A30002	Inquire
Alexa Fluor <sup>®</sup> 532	A20001	A20101
Alexa Fluor <sup>®</sup> 546	A20002	A20102
Alexa Fluor <sup>®</sup> 555	A20009	A20109
Alexa Fluor <sup>®</sup> 568	A20003	A20103
Alexa Fluor <sup>®</sup> 594	A20004	A20104
Alexa Fluor <sup>®</sup> 610-X	A30050	NA
Alexa Fluor <sup>®</sup> 633	A20005	A20105
Alexa Fluor <sup>®</sup> 647	A20006	A20106
Alexa Fluor <sup>®</sup> 660	A20007	A20107
Alexa Fluor <sup>®</sup> 680	A20008	A20108
Alexa Fluor <sup>®</sup> 700	A20010	A20110
Alexa Fluor <sup>®</sup> 750	A20011	A20111
Alexa Fluor <sup>®</sup> 790	*	*

NA = Not available. \*Alexa Fluor<sup>®</sup> 790 dye succinimidyl ester (A30051) is available in a 100 µg unit size.

## Guidelines For Use

### Reactivity of Alexa Fluor<sup>®</sup> Succinimidyl Esters

Because there is often some loss in reactivity during the packaging of the 100 µg and 1 mg unit sizes, Molecular Probes recertifies the reactivity for each lot to be ≥50% after packaging. The method for determining reactivity may not correlate directly to the actual reactivity of the Alexa Fluor<sup>®</sup> succinimidyl ester in a specific labeling reaction. In particular, the reactivity of the Alexa Fluor<sup>®</sup> 488 succinimidyl ester may be as low as 30–40%, and the amount of dye included in the reaction may need to be adjusted accordingly.

The post-packaging reactivity for each lot is printed on the certificate of analysis. The 5 mg unit sizes are packaged by weighing out, with negligible loss in reactivity; the post-packaging reactivity is equivalent to the HPLC purity reported on the product's certificate of analysis.

### Preparing the Reactive Dye Solution

Immediately before use, dissolve the succinimidyl ester in high-quality, anhydrous dimethylsulfoxide (DMSO) or dimethyl-formamide (DMF). Once reconstituted, this reactive dye solution is somewhat unstable, especially if exposed to moisture. Molecular sieves can help to eliminate water contamination in these solvents prior to mixing with the reactive dye. Although Alexa Fluor® succinimidyl esters are water soluble, they hydrolyze into the nonreactive free acid in aqueous solutions.

### Using the Alexa Fluor® Succinimidyl Esters

A generic protocol for conjugating proteins and amine-modified nucleic acids with reactive dyes is provided in the instruction manual, *Amine-Reactive Probes* (MP00143). Note that Alexa Fluor® 633 succinimidyl ester is not suitable for labeling nucleic acids. A protocol for labeling DNA using aminoallyl dUTP and Alexa Fluor® succinimidyl esters is provided in the instruction manual, *Aminoallyl dUTP* (MP21664). These manuals are available on our website at [www.invitrogen.com](http://www.invitrogen.com).

For researchers who prefer the convenience of optimized conjugation protocols, Molecular Probes also provides the Alexa Fluor® succinimidyl esters as components of several protein and nucleic labeling kits (Table 3).

**Table 3.** Alexa Fluor® protein and nucleic acid labeling kits.

Alexa Fluor® Dye	Protein Labeling Kit	Microscale Protein Labeling Kit	Monoclonal Antibody Labeling Kit	SAIVI™ Labeling Kits	ARES™ DNA Labeling Kit	Oligo-nucleotide Amine Labeling Kit
Alexa Fluor® 350	A10170		A20180			
Alexa Fluor® 430	A10171					
Alexa Fluor® 488	A10235	A30006	A20181		A21665	A20191
Alexa Fluor® 532	A10236		A20182			
Alexa Fluor® 546	A10237		A20183		A21667	
Alexa Fluor® 555	A20174	A30007	A20187		A21677	
Alexa Fluor® 568	A10238		A20184			
Alexa Fluor® 594	A10239	A30008	A20185		A21669	
Alexa Fluor® 633	A20170					
Alexa Fluor® 647	A20173	A30009	A20186	S30043, S30044	A21676	A20196
Alexa Fluor® 660	A20171					
Alexa Fluor® 680	A20172			S30039, S30041, S30045		
Alexa Fluor® 750				S30040, S30042, S30046		

**Protein Labeling Kit**—Complete, easy-to-use kit designed for three reactions, each reaction optimized to label ~1 mg of protein.

**Microscale Protein Labeling Kit**—Complete, easy-to-use kit designed for three reactions, each reaction optimized to label ~20–100 µg of protein.

**Monoclonal Antibody Labeling Kit**—Complete ready-to-use kit designed for five reactions, each reaction optimized to label ~100 µg of an IgG antibody.

**SAIVI™ Labeling Kit**—Complete ready-to-use kits for labeling proteins for small animal *in vivo* imaging (SAIVI) applications. Kits are available for three labeling reactions using samples of 0.1 mg (S30041 and S30042) or 1 mg (S30039 and S30040) with the ability to modulate DOL, or for three labeling reactions using samples in the 0.5–3 mg range that produce labeled product of the right DOL for SAIVI applications without the need for modulation (S30045 and S30046).

**ARES™ DNA Labeling Kit**—Aminoallyl dUTP is enzymatically incorporated, then the Alexa Fluor® dye is covalently attached to the amino group. Each kit provides sufficient material for 5–10 labeling reactions of 1–5 µg of DNA.

**Oligonucleotide Amine Labeling Kit**—Designed to label oligonucleotides synthesized with an amine group on the 3' or 5' end. Each kit provides sufficient materials for 3 labeling reactions of 50 µg of DNA.

## Optical Properties

The physical characteristics of the Alexa Fluor® succinimidyl esters are listed in Table 4.

**Table 4.** Physical characteristics of the Alexa Fluor® dyes.

Dye	Molecular Weight	$\lambda_{\max}$ *	Em *	$\epsilon$ †	CF <sub>280</sub> ‡	CF <sub>260</sub> §
Alexa Fluor® 350	410	346	442	19,000	0.19	0.25
Alexa Fluor® 405	1028	401	421	34,000	0.70	0.23
Alexa Fluor® 430	702	434	541	16,000	0.28	ND
Alexa Fluor® 488	643	495	519	71,000	0.11	0.30
Alexa Fluor® 500	700	502	525	71,000	0.18	0.32
Alexa Fluor® 514	714	517	542	80,000	0.18	0.31
Alexa Fluor® 532	721	532	554	81,000	0.09	0.24
Alexa Fluor® 546	~1260	554	570	112,000	0.12	0.21
Alexa Fluor® 555	~1250	555	565	150,000	0.08	0.08
Alexa Fluor® 568	792	578	603	91,300	0.46	0.45
Alexa Fluor® 594	820	590	617	90,000	0.56	0.43
Alexa Fluor® 610-X	1285	602	624	132,000	0.44	0.43
Alexa Fluor® 633 **	~1200	632	647	100,000	0.55	ND
Alexa Fluor® 647 **	~1300	650	665	239,000	0.03	0.00
Alexa Fluor® 660 **	~1100	663	690	132,000	0.10	0.00
Alexa Fluor® 680 **	~1150	679	702	184,000	0.05	0.00
Alexa Fluor® 700 **	~1400	702	723	192,000	0.07	0.00
Alexa Fluor® 750 **	~1300	749	775	240,000	0.04	0.00
Alexa Fluor® 790 **	~1750	785	810	260,000	0.08	0.09

\* Fluorescence absorbance and emission maxima, in nm, conjugated to an IgG antibody. † Extinction coefficient at  $\lambda_{\max}$  in  $\text{cm}^{-1}\text{M}^{-1}$ . ‡ Correction factor for absorption readings ( $A_{280}$ ) at 280 nm; e.g.,  $A_{280,\text{actual}} = A_{280,\text{observed}} - (\text{CF}_{280} \times \lambda_{\max})$ . § Correction factor for absorption readings ( $A_{260}$ ) at 260 nm; e.g.,  $A_{260,\text{actual}} = A_{260,\text{observed}} - (\text{CF}_{260} \times \lambda_{\max})$ . \*\* Human vision is insensitive to light beyond ~650 nm, and therefore it is not possible to view the far-red-fluorescent dyes by looking through the eyepiece of a conventional fluorescence microscope. ND = Not determined.

## Reference

1. Bioconjug Chem 6, 447 (1995).

**Product List** Current prices may be obtained from our website or from our Customer Service Department.

<b>Cat. no.</b>	<b>Product Name</b>	<b>Unit Size</b>
A10168	Alexa Fluor® 350 carboxylic acid, succinimidyl ester .....	5 mg
A30000	Alexa Fluor® 405 carboxylic acid, succinimidyl ester .....	1 mg
A30100	Alexa Fluor® 405 carboxylic acid, succinimidyl ester .....	5 mg
A10169	Alexa Fluor® 430 carboxylic acid, succinimidyl ester .....	5 mg
A20000	Alexa Fluor® 488 carboxylic acid, succinimidyl ester *mixed isomers* .....	1 mg
A20100	Alexa Fluor® 488 carboxylic acid, succinimidyl ester *mixed isomers* .....	5 mg
A30002	Alexa Fluor® 514 carboxylic acid, succinimidyl ester *mixed isomers* .....	1 mg
A20001	Alexa Fluor® 532 carboxylic acid, succinimidyl ester .....	1 mg
A20101MP	Alexa Fluor® 532 carboxylic acid, succinimidyl ester .....	5 mg
A20002	Alexa Fluor® 546 carboxylic acid, succinimidyl ester .....	1 mg
A20102	Alexa Fluor® 546 carboxylic acid, succinimidyl ester .....	5 mg
A20009	Alexa Fluor® 555 carboxylic acid, succinimidyl ester .....	1 mg
A20109	Alexa Fluor® 555 carboxylic acid, succinimidyl ester .....	5 mg
A20003	Alexa Fluor® 568 carboxylic acid, succinimidyl ester *mixed isomers* .....	1 mg
A20103	Alexa Fluor® 568 carboxylic acid, succinimidyl ester *mixed isomers* .....	5 mg
A20004	Alexa Fluor® 594 carboxylic acid, succinimidyl ester *mixed isomers* .....	1 mg
A20104	Alexa Fluor® 594 carboxylic acid, succinimidyl ester *mixed isomers* .....	5 mg
A30050	Alexa Fluor® 610-X, succinimidyl ester, bis(triethylammonium salt) *6-isomer* .....	1 mg
A20005	Alexa Fluor® 633 carboxylic acid, succinimidyl ester .....	1 mg
A20105	Alexa Fluor® 633 carboxylic acid, succinimidyl ester .....	5 mg
A20006	Alexa Fluor® 647 carboxylic acid, succinimidyl ester .....	1 mg
A20106	Alexa Fluor® 647 carboxylic acid, succinimidyl ester .....	5 mg
A20007	Alexa Fluor® 660 carboxylic acid, succinimidyl ester .....	1 mg
A20107	Alexa Fluor® 660 carboxylic acid, succinimidyl ester .....	5 mg
A20008	Alexa Fluor® 680 carboxylic acid, succinimidyl ester .....	1 mg
A20108	Alexa Fluor® 680 carboxylic acid, succinimidyl ester .....	5 mg
A20010	Alexa Fluor® 700 carboxylic acid, succinimidyl ester .....	1 mg
A20110	Alexa Fluor® 700 carboxylic acid, succinimidyl ester .....	5 mg
A20011	Alexa Fluor® 750 carboxylic acid, succinimidyl ester .....	1 mg
A20111	Alexa Fluor® 750 carboxylic acid, succinimidyl ester .....	5 mg
A30051	Alexa Fluor® 790 carboxylic acid, succinimidyl ester, penta (triethylammonium) salt .....	100 µg

## Contact Information

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