

## Alexa Fluor® Succinimidyl Esters (NHS esters)

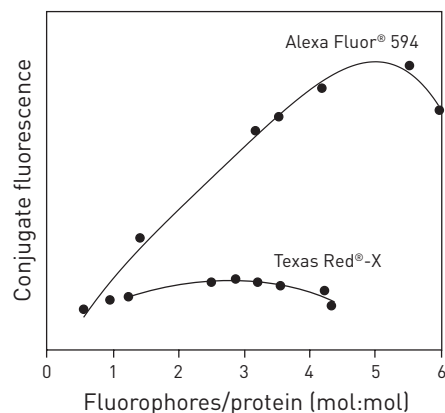
Table 1 Contents and storage

Material	Amount	Storage	Stability
Alexa Fluor® succinimidyl esters (NHS esters)	3 × 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		
	25 mg		
Approximate fluorescence excitation and emission maxima: See Table 4.			

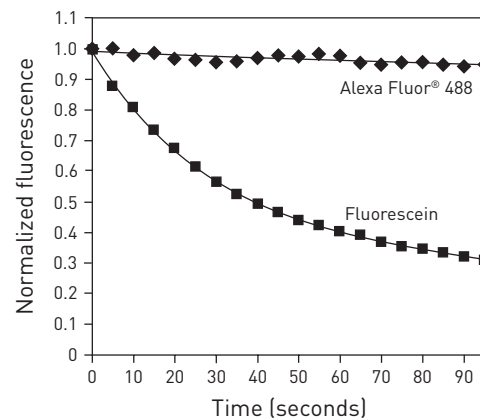
### Introduction

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 (commonly known as NHS 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. 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 (NHS esters)

Alexa Fluor <sup>®</sup> Dye	Succinimidyl Ester (NHS Ester)			
	3 × 100 µg Unit Size	1 mg Unit Size	5 mg Unit Size	25 mg Unit Size
Alexa Fluor <sup>®</sup> 350	NA	NA	A10168	NA
Alexa Fluor <sup>®</sup> 405	NA	A30000	A30100	NA
Alexa Fluor <sup>®</sup> 430	NA	NA	A10169	NA
Alexa Fluor <sup>®</sup> 488	A37570*	A20000	A20100	A37563*
Alexa Fluor <sup>®</sup> 500	NA	A30001	Inquire	NA
Alexa Fluor <sup>®</sup> 514	NA	A30002	Inquire	NA
Alexa Fluor <sup>®</sup> 532	NA	A20001	A20101	NA
Alexa Fluor <sup>®</sup> 546	NA	A20002	A20102	NA
Alexa Fluor <sup>®</sup> 555	A37571	A20009	A20109	A37564
Alexa Fluor <sup>®</sup> 568	NA	A20003	A20103	NA
Alexa Fluor <sup>®</sup> 594	A37572	A20004	A20104	A37565
Alexa Fluor <sup>®</sup> 610-X	NA	A30050	NA	NA
Alexa Fluor <sup>®</sup> 633	NA	A20005	A20105	NA
Alexa Fluor <sup>®</sup> 647	A37573	A20006	A20106	A37566
Alexa Fluor <sup>®</sup> 660	NA	A20007	A20107	NA
Alexa Fluor <sup>®</sup> 680	A37574	A20008	A20108	A37567
Alexa Fluor <sup>®</sup> 700	NA	A20010	A20110	NA
Alexa Fluor <sup>®</sup> 750	A37575	A20011	A20111	A37568
Alexa Fluor <sup>®</sup> 790	A30051**	NA	A37569**	NA

NA = Not available. \*These products are TFP esters. See "Technical Tip: Greater Control and More Reaction Time with SDP Ester" (X30052) on the product pages. \*\*Alexa Fluor<sup>®</sup> 790 dye succinimidyl ester is available in a 100 µg unit size (Cat. no. A30051) or a 10 mg unit size (Cat. no. A37569).

## Guidelines For Use

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### Reactivity of Alexa Fluor® Succinimidyl Esters

Because there is often some loss in reactivity during the packaging of the 100 µg and 1 mg unit sizes, Life Technologies 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® succinimidyl ester in a specific labeling reaction. In particular, the reactivity of the Alexa Fluor® 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 and 25 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 user guide, *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 user guide, *Aminoallyl dUTP* (MP21664). These user guides are available on our website at [www.lifetechnologies.com](http://www.lifetechnologies.com).

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

### Optical Properties

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

**Table 3** Alexa Fluor<sup>®</sup> protein and nucleic acid labeling kits

Alexa Fluor <sup>®</sup> Dye	Protein Labeling Kit	Microscale Protein Labeling Kit	APEX <sup>®</sup> Antibody Labeling Kit	Antibody Labeling Kit	SAIVI <sup>™</sup> Labeling Kit	ARES <sup>™</sup> DNA Labeling Kit	Oligonucleotide Amine Labeling Kit
Alexa Fluor <sup>®</sup> 350	A10170			A20180			
Alexa Fluor <sup>®</sup> 430	A10171						
Alexa Fluor <sup>®</sup> 488	A10235	A30006	A10468	A20181		A21665	A20191
Alexa Fluor <sup>®</sup> 532	A10236			A20182			
Alexa Fluor <sup>®</sup> 546	A10237			A20183		A21667	
Alexa Fluor <sup>®</sup> 555	A20174	A30007	A10470	A20187		A21677	
Alexa Fluor <sup>®</sup> 568	A10238		A10494	A20184			
Alexa Fluor <sup>®</sup> 594	A10239	A30008	A10474	A20185		A21669	
Alexa Fluor <sup>®</sup> 633	A20170						
Alexa Fluor <sup>®</sup> 647	A20173	A30009	A10475	A20186	S30044	A21676	A20196
Alexa Fluor <sup>®</sup> 660	A20171						
Alexa Fluor <sup>®</sup> 680	A20172			A20188	S30045		
Alexa Fluor <sup>®</sup> 750					S30046		
Alexa Fluor <sup>®</sup> 790				A20189			

**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.

**APEX<sup>®</sup> Antibody Labeling Kit**—Complete, ready-to-use kit designed for five reactions, each reaction optimized to label small amounts of IgG antibody (~10–20 µg). It is ideal for the efficient labeling of antibodies in serum, ascites fluid, or hybridoma suspensions.

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

**SAIVI<sup>™</sup> 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<sup>™</sup> DNA Labeling Kit**—Aminoallyl dUTP is enzymatically incorporated, then the Alexa Fluor<sup>®</sup> 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.

**Table 4** Physical characteristics of the Alexa Fluor<sup>®</sup> dyes

Dye	Molecular Weight	$\lambda_{\max}^*$	$\text{Em}^*$	$\epsilon^\dagger$	$\text{CF}_{280}^\ddagger$	$\text{CF}_{260}^\S$
Alexa Fluor <sup>®</sup> 350	410	346	442	19,000	0.19	0.25
Alexa Fluor <sup>®</sup> 405	1028	401	421	34,000	0.70	0.23
Alexa Fluor <sup>®</sup> 430	702	434	541	16,000	0.28	ND
Alexa Fluor <sup>®</sup> 488	643	495	519	71,000	0.11	0.30
Alexa Fluor <sup>®</sup> 500	700	502	525	71,000	0.18	0.32
Alexa Fluor <sup>®</sup> 514	714	517	542	80,000	0.18	0.31
Alexa Fluor <sup>®</sup> 532	721	532	554	81,000	0.09	0.24
Alexa Fluor <sup>®</sup> 546	~1260	554	570	112,000	0.12	0.21
Alexa Fluor <sup>®</sup> 555	~1250	555	565	150,000	0.08	0.08
Alexa Fluor <sup>®</sup> 568	792	578	603	91,300	0.46	0.45
Alexa Fluor <sup>®</sup> 594	820	590	617	90,000	0.56	0.43
Alexa Fluor <sup>®</sup> 610-X	1285	602	624	132,000	0.44	0.43
Alexa Fluor <sup>®</sup> 633**	~1200	632	647	100,000	0.55	ND
Alexa Fluor <sup>®</sup> 647**	~1300	650	665	239,000	0.03	0.00
Alexa Fluor <sup>®</sup> 660**	~1100	663	690	132,000	0.10	0.00
Alexa Fluor <sup>®</sup> 680**	~1150	679	702	184,000	0.05	0.00
Alexa Fluor <sup>®</sup> 700**	~1400	702	723	192,000	0.07	0.00
Alexa Fluor <sup>®</sup> 750**	~1300	749	775	240,000	0.04	0.00
Alexa Fluor <sup>®</sup> 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  $\text{cm}^2\text{M}^{-1}$ . ‡ 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 absorbance 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.

Cat. no.	Product Name	Unit Size
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
A37571	Alexa Fluor® 555 NHS ester, tris(triethylammonium salt)	3 × 100 µg
A20009	Alexa Fluor® 555 carboxylic acid, succinimidyl ester	1 mg
A20109	Alexa Fluor® 555 carboxylic acid, succinimidyl ester	5 mg
A37564	Alexa Fluor® 555 NHS ester, tris (triethylammonium salt)	25 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
A37572	Alexa Fluor® 594 NHS ester, tris(triethylammonium salt)	3 × 100 µg
A20004	Alexa Fluor® 594 carboxylic acid, succinimidyl ester *mixed isomers*	1 mg
A20104	Alexa Fluor® 594 carboxylic acid, succinimidyl ester *mixed isomers*	5 mg
A37565	Alexa Fluor® 594 NHS ester, tris (triethylammonium salt)	25 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
A37573	Alexa Fluor® 647 NHS ester, tris(triethylammonium salt)	3 × 100 µg
A20006	Alexa Fluor® 647 carboxylic acid, succinimidyl ester	1 mg
A20106	Alexa Fluor® 647 carboxylic acid, succinimidyl ester	5 mg
A37566	Alexa Fluor® 647 NHS ester, tris (triethylammonium salt)	25 mg
A20007	Alexa Fluor® 660 carboxylic acid, succinimidyl ester	1 mg
A20107	Alexa Fluor® 660 carboxylic acid, succinimidyl ester	5 mg
A37574	Alexa Fluor® 680 NHS ester, bis (triethylammonium salt)	3 × 100 µg
A20008	Alexa Fluor® 680 carboxylic acid, succinimidyl ester	1 mg
A20108	Alexa Fluor® 680 carboxylic acid, succinimidyl ester	5 mg
A37567	Alexa Fluor® 680 NHS ester, bis (triethylammonium salt)	25 mg
A20010	Alexa Fluor® 700 carboxylic acid, succinimidyl ester	1 mg
A20110	Alexa Fluor® 700 carboxylic acid, succinimidyl ester	5 mg
A37575	Alexa Fluor® 750 NHS ester, tris(triethylammonium salt)	3 × 100 µg
A20011	Alexa Fluor® 750 carboxylic acid, succinimidyl ester	1 mg
A20111	Alexa Fluor® 750 carboxylic acid, succinimidyl ester	5 mg
A37568	Alexa Fluor® 750 NHS ester, tris (triethylammonium salt)	25 mg
A30051	Alexa Fluor® 790 carboxylic acid, succinimidyl ester, penta (triethylammonium) salt	100 µg
A37569	Alexa Fluor® 790 NHS ester, penta (triethylammonium salt)	10 mg

# Purchaser Notification

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Additional international offices are listed at  
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These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

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