PRODUCT INFORMATION

iFluor® 594 succinimidyl ester

Item No. 44058

Synoynm: iFluor® 594 SE
FW: 1,160.4
Ex./Em. Max.: 587/603 nm
Supplied as: A solid
Storage: -20°C
Stability: ≥4 years
Special Conditions: Light sensitive

Information represents the product specifications. Batch specific analytical results are provided on each certificate of analysis.

Laboratory Procedures

iFluor® 594 succinimidyl ester (iFluor® 594 SE) is supplied as a solid. A stock solution may be made by dissolving the iFluor® 594 SE in the solvent of choice, which should be purged with an inert gas. iFluor® 594 SE is soluble in DMSO.

Description

iFluor® 594 SE is an amine-reactive fluorescent probe. It displays excitation/emission maxima of 587/603 nm, respectively. iFluor® 594 SE can be used to label primary amines on proteins and antibodies.

Assay Protocol

NOTE: Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20°C after preparation. Avoid repeated freeze-thaw cycles.

Prepare Protein Labeling Stock Solution (Solution A)

1. Mix 100 µl of reaction buffer (*e.g.*, 1 M sodium bicarbonate or 1 M phosphate buffer with pH ~9) with 900 µl of the target protein solution (*e.g.*, antibody or protein with a concentration of >2 mg/ml, if possible).

NOTE 1: The pH of Solution A should be 8.5 ± 0.5. If the pH of Solution A is lower than 8, adjust the pH to the range of 8-9 using 1 M sodium bicarbonate or 1 M phosphate buffer, pH 9.

NOTE 2: The protein should be dissolved in PBS (1X), pH 7.2-7.4. If the protein is dissolved in a Tris or glycine buffer, it must be dialyzed against PBS (1X), pH 7.2-7.4, to remove free amines or ammonium salts, such as ammonium sulfate or ammonium acetate, which are commonly used for protein precipitation.

NOTE 3: Impure antibodies or antibodies stabilized with BSA or gelatin will not be labeled well. The presence of sodium azide or thimerosal (Item No. 33432) might also interfere with the conjugation reaction. Sodium azide or thimerosal can be removed by dialysis or using a spin column for optimal labeling results.

NOTE 4: The conjugation efficiency is significantly reduced if the protein concentration is less than 2 mg/ml. The final protein concentration range of 2-10 mg/ml is recommended for optimal labeling efficiency.

Prepare iFluor® 594 SE Stock Solution (Solution B)

1. Prepare a 10 mM stock solution of iFluor® 594 SE by adding anhydrous DMSO to the vial. Mix well by pipetting or vortex.

WARNING
THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

SAFETY DATA
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NOTE: Prepare Solution B before starting the conjugation. Use promptly. Extended storage of Solution B may reduce dye activity. Solution B may be stored at -20°C for up to two weeks when protected from light and moisture. Avoid repeated freeze-thaw cycles.

Sample Experimental Protocol

This labeling protocol was developed for the labeling of goat anti-mouse IgG with iFluor® 594 SE. Further optimization may be required for your specific proteins.

NOTE: Each protein requires a distinct dye:protein ratio, which also depends on the properties of dyes. Over-labeling of a protein could detrimentally affect its binding affinity while the protein conjugates of low dye:protein ratio give reduced sensitivity.

Run Conjugation Reaction

1. Use a 10:1 molar ratio of Solution B (dye):Solution A (protein) as the starting point. Add 5 µl of the dye stock solution (Solution B, assuming the dye stock solution is 10 mM) into the vial of the protein solution (95 µl of Solution A) with effective shaking. The concentration of the protein is ~0.05 mM assuming the protein concentration is 10 mg/ml and the molecular weight of the protein is ~200 KDa.

NOTE: We recommend using a 10:1 molar ratio of dye to protein. If the ratio is too low or too high, determine the optimal dye:protein ratio at 5:1, 15:1, and 20:1, respectively.

2. Continue to rotate or shake the reaction mixture at room temperature for 30-60 minutes.

Purify the Conjugation

The following protocol is an example of dye-protein conjugate purification using a Sephadex G-25 column.

1. Prepare Sephadex G-25 column according to the manufacture's instructions.
2. Load the reaction mixture (from "Run conjugation reaction") to the top of the Sephadex G-25 column.
3. Add PBS (pH 7.2-7.4) as soon as the sample runs just below the top resin surface.
4. Add more PBS (pH 7.2-7.4) to the desired sample to complete the column purification. Combine the fractions that contain the desired dye-protein conjugate.

NOTE: For immediate use, the dye-protein conjugate needs to be diluted with staining buffer, and aliquoted for multiple uses.

NOTE: For longer term storage, the dye-protein conjugate solution needs to be concentrated or freeze-dried.

Characterize the Desired Dye-Protein Conjugate

The Degree of Substitution (DOS) is the most important factor for characterizing dye-labeled protein. Proteins of lower DOS usually have weaker fluorescence intensity, but proteins of higher DOS tend to have reduced fluorescence too. The optimal DOS for most antibodies is recommended between 2 and 10 depending on the properties of dye and protein. For effective labeling, the DOS should be controlled to have 5-8 moles of iFluor® 594 SE to one mole of antibody. The following steps are used to determine the DOS of iFluor® 594 SE labeled proteins.

Measure absorption

To measure the absorption spectrum of a dye-protein conjugate, it is recommended to keep the sample concentration in the range of 1-10 µM, depending on the extinction coefficient of the dye.

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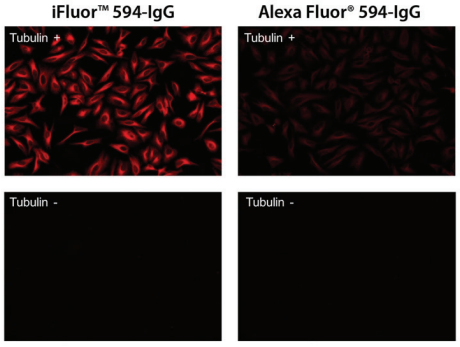
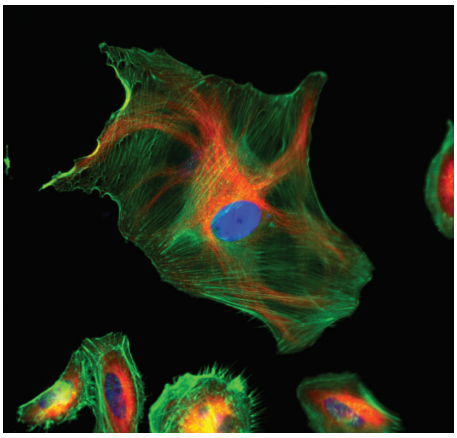
Read OD (absorbance) at 280 nm and dye maximum absorption (λ_{max} = 588 nm for iFluor™ 594 dyes)
For most spectrophotometers, the sample (from the column fractions) needs to be diluted with deionized water so that the OD values are in the range of 0.1 to 0.9. The O.D. (absorbance) at 280 nm is the maximum absorption of protein while 588 nm is the maximum absorption of iFluor® 594 SE. To obtain accurate DOS, make sure that the conjugate is free of the non-conjugated dye.

Calculate DOS

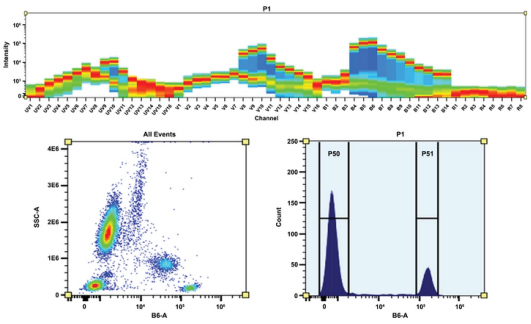
Images



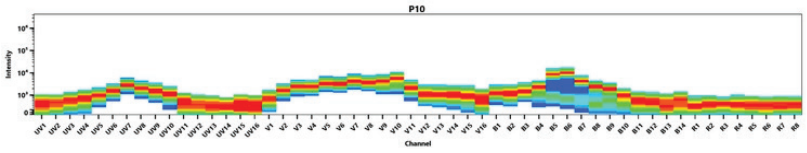
HeLa cells were stained with (Tubulin+) or without (Tubulin-) mouse anti-tubulin and then visualized with iFluor® 594 goat anti-mouse IgG (Left) or with Alexa Fluor® 594 goat anti-mouse IgG (Right).



HeLa cells were stained with mouse anti-tubulin followed with iFluor® 594 goat anti-mouse IgG (H+L) (red). Actin filaments were stained with Phalloidin-iFluor® 488 conjugate (green) and nuclei were stained with DAPI (blue).



Top: Spectral pattern was generated using a 4-laser spectral cytometer. Spatially offset lasers (355 nm, 405 nm, 488 nm, and 640 nm) were used to create four distinct emission profiles, then, when combined, yielded the overall spectral signature.
Bottom: Flow cytometry analysis of whole blood cells stained with PE/iFluor® 594 anti-human CD4 SK3 conjugate. The fluorescence signal was monitored using an Aurora spectral flow cytometer in the PE/iFluor® 594 specific B6-A channel.



Spectral signature of iFluor® 594 dye. Data acquired on a 4-laser Cytek Aurora and normal human peripheral blood cells stained with clone SK3 (CD4) conjugated to iFluor® 594 dye were used for analysis.

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