

PRODUCT INFORMATION



iFluor® 405 succinimidyl ester

Item No. 44056

Synonym: iFluor® 405 SE

FW: 755.6

Ex./Em. Max.: 403/427 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® 405 succinimidyl ester (iFluor® 405 SE) is supplied as a solid. A stock solution may be made by dissolving the iFluor® 405 SE in the solvent of choice, which should be purged with an inert gas. iFluor® 405 SE is soluble in DMSO.

Description

iFluor® 405 SE is an amine-reactive fluorescent probe. It displays excitation/emission maxima of 403/427 nm, respectively. iFluor® 405 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. 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.

WARNING

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

SAFETY DATA

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent via email to your institution.

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Prepare iFluor® 405 SE Stock Solution (Solution B)

1. Prepare a 10 mM stock solution of iFluor® 405 SE by adding anhydrous DMSO to the vial. Mix well by pipetting or vortex.

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® 405 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 a 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 by using a Sephadex G-25 column.

1. Purify the conjugate on a gel filtration column, such as a Sephadex G-25 column or equivalent matrix, or by extensive dialysis at 4°C in an appropriate buffer.

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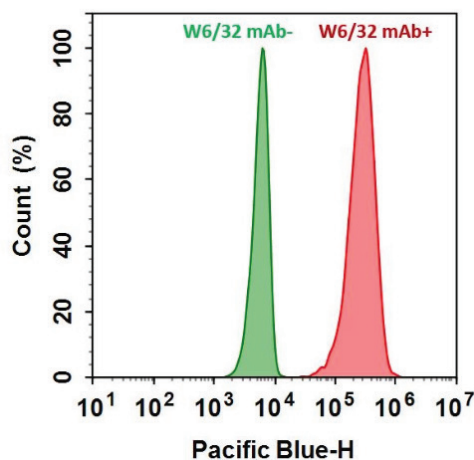


Optional: Characterize the Desired Dye-Protein Conjugate

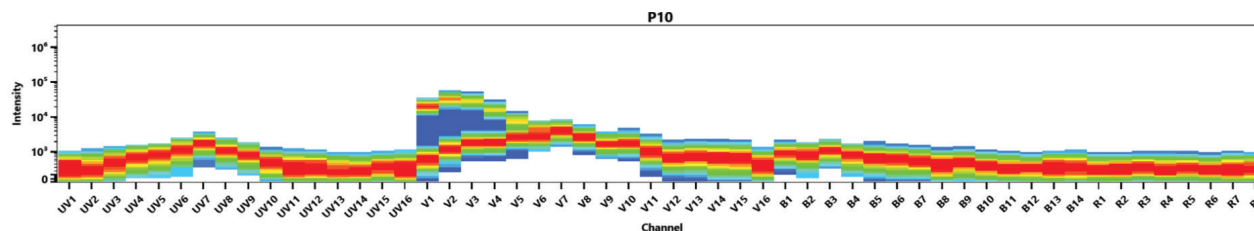
Determining the Degree of Substitution (DOS) is crucial in characterizing dye-labeled proteins. Lower DOS proteins tend to have weaker fluorescence, but higher DOS proteins may also have reduced fluorescence. For most antibodies, the optimal DOS is between 2 and 10, depending on the dye and protein properties. For effective labeling, the degree of substitution should be controlled to have 5-8 moles of iFluor® 405 SE to one mole of antibody. The following steps are used to determine the DOS of iFluor® 405 SE-labeled proteins:

1. Measure absorption— To measure the absorption spectrum of a dye-protein conjugate, the sample concentration should be kept between 1 and 10 μM , depending on the dye's extinction coefficient.
2. Read OD (absorbance) at 280 nm and dye maximum absorption (λ_{max} = 403 nm for iFluor® 405 SE dyes). For most spectrophotometers, the sample (from the column fractions) must be diluted with de-ionized water so that the OD values range from 0.1 to 0.9. The O.D. (absorbance) at 280 nm is the maximum absorption of protein, while 403 nm is the maximum absorption of iFluor® 405 SE. To obtain accurate DOS, ensure the conjugate is free of the non-conjugated dye.
3. Calculate DOS.

Images



HL-60 cells were incubated with (red, +) or without (green, -) Anti-human HLA-ABC (W6/32 mAb), followed by iFluor® 405 goat anti-mouse IgG (H&L). The fluorescence signal was monitored using ACEA NovoCyt flow cytometer in the Pacific Blue channel (Ex/Em=405/445 nm).



Spectral signature of iFluor® 405 dye. Data acquired on a 4-laser Cytex Aurora and normal human peripheral blood cells stained with clone SK3 (CD4) conjugated to iFluor® 405 dye were used for analysis.

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