ICG-Sulfo-OSu

Catalog number: 180
Unit size: 1 mg

Component	Storage	Amount (Cat No. 180)
ICG-Sulfo-OSu	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)

OVERVIEW

Indocyanine green (ICG) is a cyanine dye used in medical diagnostics. It is used for determining cardiac output, hepatic function, and liver blood flow, and for ophthalmic angiography. It has a peak spectral absorption close to 800 nm. These infrared frequencies penetrate retinal layers, allowing ICG angiography to image deeper patterns of circulation than fluorescein angiography. ICG binds tightly to plasma proteins and becomes confined to the vascular system. ICG has a half-life of 150 to 180 seconds and is removed from circulation exclusively by the liver to bile juice. A recent study indicated ICG targets atheromas within 20 min of injection and provides sufficient signal enhancement for in vivo detection of lipid-rich, inflamed, coronary-sized plaques in atherosclerotic rabbits. Ex vivo fluorescence reflectance imaging showed high plaque target-to-background ratios in atheroma-bearing rabbits injected with ICG compared to atheroma-bearing rabbits injected with saline. This amino-reactive ICG derivative is used to make ICG bioconjugates with antibodies and other biological molecules. It has moderate water solubility.

AT A GLANCE

Molecular Weight: 930.07
Solvent: DMSO
Extinction Coefficient: 230,000 cm-1M-1
Excitation/Emission: 780/800 nm
CF at 260/280 nm: 0.113/0.073

Important Note

Extinction coefficient are at their maximum absorption wavelength. CF at 260 nm is the correction factor used for eliminating the dye contribution to the absorbance at 260 nm (for oligo and nucleic acid labeling). CF at 280 nm is the correction factor used for eliminating the dye contribution to the absorbance at 280 nm (for peptide and protein labeling). Fluorescence intensity is significantly increased upon coupled to proteins. This labeling protocol was developed for the conjugate of Goat anti-mouse IgG with ICG. You might need further optimization for your particular proteins.

PREPARATION OF STOCK SOLUTIONS

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

Protein Stock Solution (Solution A)

Mix 100 µL of a reaction buffer (e.g., 1 M sodium carbonate solution or 1 M phosphate buffer with pH ~9.0) with 900 µL of the target protein solution (e.g. antibody, protein concentration ≥2 mg/ml if possible) to give 1 mL protein labeling stock solution. The pH of the protein solution should be 8.5 ± 0.5. If the pH of the protein solution is lower than 8.0, adjust the pH to the range of 8.0 - 9.0 using 1 M sodium bicarbonate solution or 1 M pH 9.0 phosphate buffer. The protein should be dissolved in 1X phosphate buffered saline (PBS), pH 7.2 - 7.4. If the protein is dissolved in Tris or glycine buffer, it must be dialyzed against 1X PBS, pH 7.2 - 7.4, to remove free amines or ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for protein precipitation. Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well. The presence of sodium azide or thimerosal might also interfere with the conjugation reaction. Sodium azide or thimerosal can be removed by dialysis or spin column for optimal labeling results. For optimal labeling efficiency, the final protein concentration range of 2 - 10 mg/mL is recommended, with the conjugation efficiency significantly reduced if less than 2 mg/mL.

Dye Stock Solution (Solution B)

Add anhydrous DMSO into the vial of ICG dyes to make a 10 - 20 mM stock solution. Mix well by pipetting or vortex. Prepare the dye stock solution before starting the conjugation. Use promptly. Extended storage of the dye stock solution may reduce dye activity.

SAMPLE EXPERIMENTAL PROTOCOL

Determine the optimal dye/protein ratio (optional):

1. Use 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 ~200KD. The concentration of the DMSO in the protein solution should be <10%.
2. Run conjugation reaction.
3. Repeat Step 2 with the molar ratios of Solution B/Solution A at 5:1; 15:1 and 20:1 respectively.
4. Purify the desired conjugates using premade spin columns.
5. Calculate the dye/protein ratio (DOS) for the above 4 conjugates (if step 3 is done).
6. Run your functional tests of the above 4 conjugates to determine the best dye/protein ratio to scale up your labeling reaction.

Run conjugation reaction:

1. Add the appropriate amount of dye stock solution (Solution B) into the vial of the protein solution (Solution A) with effective shaking. The best molar ratio of Solution B/Solution is determined above. If skipped, we recommend using 10:1 molar ratio of Solution B (dye)/Solution A (protein).
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. Prepare Sephadex G-25 column according to the manufacture instruction.
2. Load the reaction mixture 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. For immediate use, the dye-protein conjugate need be diluted with staining buffer, and aliquoted for multiple uses. For longer term storage, dye-protein conjugate solution need be concentrated or freeze dried.

EXAMPLE DATA ANALYSIS AND FIGURES

The Degree of Labeling (DOL) is the most important factor for characterizing dye-labeled proteins. Proteins of lower DOL usually have weaker fluorescence intensity, but proteins of higher DOL (e.g. DOL >6) tend to have reduced fluorescence too. The optimal DOL for most antibodies is recommended between 2 and 10 depending on the properties of dye and protein. For effective labeling, the degree of substitution should be controlled to have 4 - 10 moles of ICG to one mole of antibody.

Below is the protocol for calculating the DOL of ICG labeled proteins.For convenience, use the Degree of Labeling Calculator: https://www.aatbio.com/tools/degree-of-labeling-calculator/

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.

Read OD (absorbance) at 280 nm and dye maximum absorption (λ_{max} = 785 nm for ICG dyes)

For most spectrophotometers, the sample (from the column fractions) need be diluted with de-ionized water so that the OD values are in the range of 0.1 to 0.9. The O.D. at 280 nm is the maximum absorption of proteins while 785 nm is the maximum absorption of ICG dyes. To obtain accurate DOL, make sure that the conjugate is free of the non-conjugated dyes.

Calculate DOL using the following equations

Calculate protein concentration [Protein] = (A_{280} - (OD_{785} x CF_{280}))/Protein Extinction Coefficient x dilution factor

Calculate dye concentration [Dye] = (OD_{785}/230,000) x dilution factor

Calculate the degree of labeling DOL = [Dye]/[Protein] = [$^{D}OD_{785}$ x $^{P}\varepsilon_{280}$] /[230,000 × (A_{280} - 0.073 x A_{785})]

[Dye] is the dye concentration, and can be readily calculated from the Beer-Lambert Law: $A=\varepsilon_{dye}CL$. [Protein] is the protein concentration, either estimated by the weight (added to the reaction) if the conjugation efficiency is high enough (preferably > 70%) or more accurately calculated by the Beer-Lambert Law: $A=\varepsilon_{protein}CL$. $^{P}\varepsilon_{280}$ = protein molar extinction coefficient at 280 nm (e.g. the molar extinction coefficient of IgG is 203,000 $cm^{-1}M^{-1}$). CF (dye absorption correction factor at 280 nm) = OD_{280}/OD_{750} = 0.073 for ICG-Sulfo-OSu. 230,000 $cm^{-1}M^{-1}$ is the molar extinction coefficient of ICG-Sulfo-OSu.

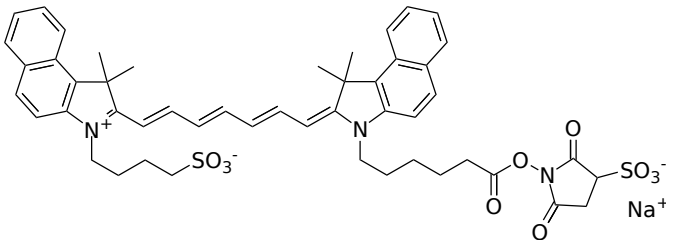

Figure 1. Chemical structure for ICG-Sulfo-OSu

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