Revised: 11–January–2001

SAMSA Fluorescein (A-685)

Quick Facts

Storage upon receipt:
- –20°C
- Desiccate
- Protect from light

Molecular weight: 521

Introduction

Molecular Probes' 5-((2-(and-3)-*S*-(acetylmercapto)succinoyl)amino)fluorescein (SAMSA fluorescein, A-685) is a useful reagent for forming fluorescent protein conjugates and for assaying maleimide and iodoacetamide moieties on proteins. SAMSA fluorescein is activated with base to remove the acetyl protecting group, thereby generating thiol-containing fluorescein. Chamow and colleagues employed activated SAMSA fluorescein to produce a fluorescent conjugate of the soluble form of glycoprotein CD4 (sCD4).[1] By using a novel heterobifunctional crosslinking reagent, they were able to fluorescently label the carbohydrate moieties of sCD4 without affecting its affinity for the HIV-1 envelope protein gp120.

Storage and Handling

SAMSA fluorescein, with a molecular weight of 521, is supplied as lyophilized powder in units of 25 mg. This solid should be stored desiccated at -20°C and protected from light. Solutions of SAMSA fluorescein can be made by dissolving the powder in dimethylformamide or in buffer (pH >6); storage in aqueous solution is not recommended.

Application

SAMSA Fluorescein Activation

Dissolve 10 mg SAMSA fluorescein in 1 mL 0.1 M NaOH and incubate at room temperature for 15 minutes to remove the acetyl protecting group. Following activation of SAMSA fluorescein, the solution should be neutralized with concentrated HCl (approximately 14 µL of 6 M HCl) and buffered with 0.2 mL of 0.5 M sodium phosphate, pH 7. Activate only as much SAMSA fluorescein reagent as needed for subsequent reaction, as the excess thiol-containing fluorescein will be oxidized to the disulfide over time. Storage of the frozen solution under nitrogen or argon may prolong the life of the thiol.

Protein Conjugation

Incubate the activated SAMSA fluorescein with the thiol-reactive protein at room temperature for approximately 30 minutes. Note that you must first remove any excess maleimide from the thiol-reactive protein preparation or use enough activated SAMSA fluorescein to consume all thiol-reactive reagents. We recommend that activated SAMSA fluorescein be added at a 5–10-fold molar excess to the thiol-reactive moieties present in the reaction mixture. Following conjugation, the unreacted dye can be separated from the labeled protein using a Sephadex® G-25 gel filtration column equilibrated with phosphate-buffered saline (PBS).

Determination of the Degree of Labeling

Excitation and emission maxima of SAMSA fluorescein are 495 nm and 520 nm, respectively. The degree of labeling can be determined from the absorbance of the labeled protein at 495 nm; the extinction coefficient of SAMSA fluorescein is approximately 80,000 $cm^{-1}M^{-1}$ at 495 nm.

References

1. J Biol Chem 267, 15916 (1992).

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

Cat #	Product Name	Unit Size
A-685	5-((2-(and-3)-*S*-(acetylmercapto)succinoyl)amino)fluorescein (SAMSA fluorescein) *mixed isomers*	25 mg

Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

Please visit our Web site — **www.probes.com** — for the most up-to-date information

Molecular Probes, Inc.
PO Box 22010, Eugene, OR 97402-0469
Phone: (541) 465-8300 ● Fax: (541) 344-6504

Customer Service: 7:00 am to 5:00 pm (Pacific Time)
Phone: (541) 465-8338 ● Fax: (541) 344-6504 ● order@probes.com

Toll-Free Ordering for USA and Canada:
Order Phone: (800) 438-2209 ● Order Fax: (800) 438-0228

Technical Assistance: 8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 465-8353 ● Fax: (541) 465-4593 ● tech@probes.com

Molecular Probes Europe BV
PoortGebouw, Rijnsburgerweg 10
2333 AA Leiden, The Netherlands
Phone: +31-71-5233378 ● Fax: +31-71-5233419

Customer Service: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5236850 ● Fax: +31-71-5233419
eurorder@probes.nl

Technical Assistance: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5233431 ● Fax: +31-71-5241883
eurotech@probes.nl

Molecular Probes' products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.