**FluoReporter® Cell-Surface Biotinylation Kit (F-20650)**

**Introduction**

Biotin-XX sulfosuccinimidyl ester is a cell-impermeant, amine-reactive compound that can be used to label proteins exposed on the surface of live cells (Figure 1). The sulfosuccinimidyl ester forms an extremely stable conjugate with cell-surface proteins, and the biotin provides a convenient hapten for subsequent isolation or analysis with an avidin-based protein, including streptavidin, NeutrAvidin™ or CaptAvidin™ biotin-binding proteins. Cell-surface biotinylation techniques have been employed to differentially label proteins in the apical and basolateral plasma membranes of epithelial cells. The technique is also suited to the study of internalization of membrane proteins and cell-surface targeting of proteins.

The FluoReporter® Cell-Surface Biotinylation Kit provides a convenient method to label proteins exposed on the cell surface including, but not limited to, membrane proteins. The kit contains five vials of the biotin-XX sulfosuccinimidyl ester and anhydrous DMSO for preparation of stock solutions. The supplied protocol for cell-surface biotinylation is easy to perform, and can be completed in less than one hour.

**Materials**

**Kit Contents**

- Biotin-XX, sulfosuccinimidyl ester (Component A), 5 vials, each containing 50 µg
- Dimethylsulfoxide (DMSO), anhydrous (Component B), 1.5 mL

**Storage and Handling**

Upon receipt, components should be stored desiccated at -20°C until required for use. When stored properly, both kit components should be stable for at least six months.

**Experimental Protocol**

The following protocol has been optimized for labeling of cells in suspension. For biotinylation of cells grown in monolayers, please refer to the excellent protocol given in reference 2.

---

**Figure 1.** Identification of cell-surface proteins in Jarkat cells labeled with the FluoReporter® Cell-Surface Biotinylation Kit. The labeled cells were fractionated by differential detergent extraction into membrane and cytosolic fractions. The proteins were then acetone precipitated, separated on an SDS-polyacrylamide gel and blotted onto a PVDF membrane. Using the Pro-Q™ Western Blot Stain Kit #6 (P-21862), total proteins and biotinylated proteins were differentially stained. Total proteins were detected with the SYPRO Ruby protein blot stain component of the kit and UV excitation (Panel A), while biotinylated proteins were identified with streptavidin–alkaline phosphatase, in combination with the red fluorogenic substrate, DDAO phosphate and acquired using a laser scanner equipped with 633 nm excitation (Panel B). As expected, cell-surface proteins were found only in the membrane fraction and not in the cytosolic fraction, and only a subset of the membrane proteins were found to be exposed on the cell surface. The lanes labeled “MW” contain protein molecular weight markers, and the lanes labeled “Con A” contain biotinylated concanavalin A.
1.1 Warm one vial of biotin-XX SSE (Component A) and the vial of DMSO (Component B, if it is to be used) to room temperature before opening.

1.2 Prepare a 0.2 mg/mL solution of biotin-XX SSE by dissolving the contents of the vial in either 250 µL of DMSO (Component B) or 250 µL of PBS. The solution, if in PBS, must be used immediately.

**Cell-Surface Labeling**

2.1 Wash the cells three times in ice cold PBS to remove any contaminating proteins.

2.2 Suspend the cells at a concentration of $2.5 \times 10^7$ cells/mL in PBS.

2.3 Add 2.5 µL of the biotin-XX SSE stock solution (prepared in step 1.2) per 1 mL volume of suspended cells and mix well.

2.4 Incubate the cells on ice for 30 minutes.

2.5 Wash the cells three times with ice-cold PBS to remove any unreacted biotin-XX SSE.

2.6 Prepare extracts for analysis as desired (see References and Figure 1).

**References**


**Product List**

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Product Name</th>
<th>Unit</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-20650</td>
<td>FluoReporter Cell-Surface Biotinylation Kit</td>
<td>1 kit</td>
<td>1 kit</td>
</tr>
</tbody>
</table>

**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](http://www.probes.com) — for the most up-to-date information.

**Molecular Probes, Inc.**

29851 Willow Creek Road, 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

euorder@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.

Several of Molecular Probes’ products and product applications are covered by U.S. and foreign patents and patents pending. Our products are not available for resale or other commercial uses without a specific agreement from Molecular Probes, Inc. We welcome inquiries about licensing the use of our dyes, trademarks or technologies. Please submit inquiries by e-mail to busdev@probes.com. All names containing the designation ® are registered with the U.S. Patent and Trademark Office.

Copyright 2002, Molecular Probes, Inc. All rights reserved. This information is subject to change without notice.