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

Quick Facts

Storage upon receipt:

- – 20°
- Desiccate

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 sulfosuccinidimidyl ester forms an extremely stable conjugate [1] 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.[2] Cell-surface biotinylation techniques have been employed to differentially label proteins in the apical and basolateral plasma membranes of epithelial cells.[3,4] The technique is also suited to the study of internalization of membrane proteins [2] and cell-surface targeting of proteins.[5-7]

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.

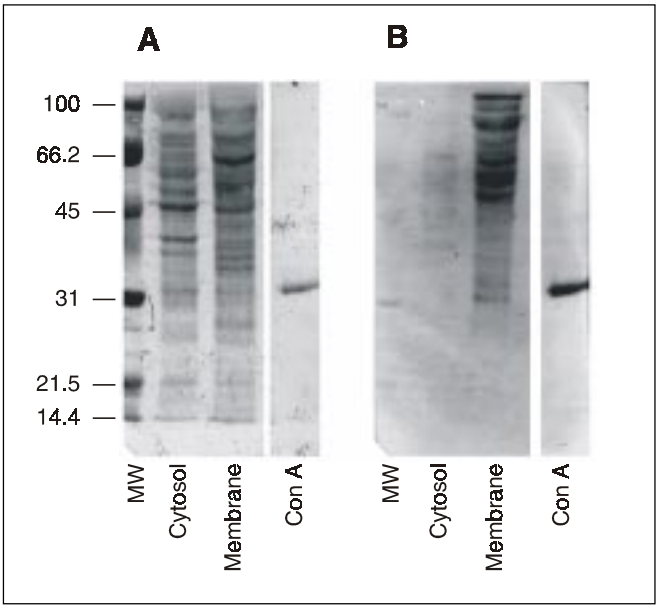

Figure 1. *Identification of cell-surface proteins in Jurkat cells labeled with the FluoReporter® Cell-Surface Biotinylation Kit. The labeled cells were fractionated by differential detergent extraction into membrane and cytosolic fractions. The proteins were then acetone precipitated, separated on an 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.*

Reagent Preparation

For convenience, the reactive biotin-XX sulfosuccinimidyl ester (biotin-XX SSE) in each kit is provided in five separate vials. Each vial provides sufficient material for labeling several cell samples. However, once reconstituted, solutions of the reagent are somewhat unstable. Biotin-XX SSE can be dissolved in either DMSO (Component B) or in phosphate-buffered saline (PBS, not provided). It is essential to use PBS stock solutions immediately. DMSO stock solutions are best used immediately, but unused portions may still be useful for up to 2 weeks if stored at -20°C, protected from moisture.

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 2.5×10^7 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

1. Bioconjugate Chem 6, 447 (1995); **2.** *Cell Biology: A Laboratory Handbook 2nd Edition*, J. Celis, Ed., pp. 341–350, Academic Press (1998); **3.** J Neurochem 77, 1301 (2001); **4.** J Cell Sci 109, 3025 (1996); **5.** J Cell Biol 153, 957 (2001); **6.** J Virol 75, 4744 (2001); **7.** J Biol Chem 274, 36801 (1999).

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

Cat #	Product Name	Unit Size
F-20650	FluoReporter® Cell-Surface Biotinylation Kit	1 kit

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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.

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