

Biotinylation of Cell Surface Proteins

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1. Cool cells on ice.
2. Wash cells three times with ice-cold PBS containing 1 mM MgCl₂ and 0.1 mM CaCl₂ (PBS/CM) to remove any contaminating proteins.
3. Suspend the cells at a concentration of 25x10⁶ cells/ml in ice-cold PBS/CM.
4. Add 0.5 mg of Sulfo-NHS-LC-Biotin (Sigma) per ml of reaction volume.
5. Incubate at room temperature for 30 min.
6. Wash cells once with ice-cold PBS/CM and quench by incubation with 0.1 M glycine, followed by three washes in ice-cold PBS.
7. Cells were harvested in modified RIPA buffer.
8. Centrifuge at 14,000 rpm for 15 min at 4c.
9. The resulting supernatant was incubated with 100 ul of 50% streptavidin agarose (Molecular Probes, Eugene, Oregon) with rotating overnight at 4c.
10. After the beads was washed five times with RIPA buffer, bound proteins were eluted with SDS sample buffer by boiling for 5 min.
11. Total protein and isolated biotinylated proteins were analyzed by immunoblotting.