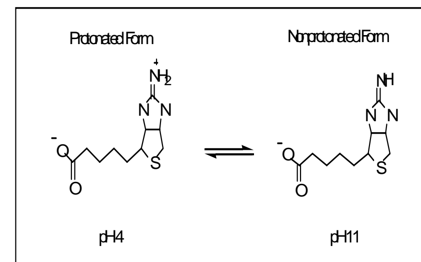
A reversible in-gel detection system for fluorescence-based analysis of plasma membrane proteins and their subsequent identification by mass spectrometry

M. O'Malley, N. Jiang, Y. Wang, H. Lisoukov, T. Miller, S. Kashi and W.F. Patton PerkinElmer Life and Analytical Sciences, Boston, MA, USA

Introduction

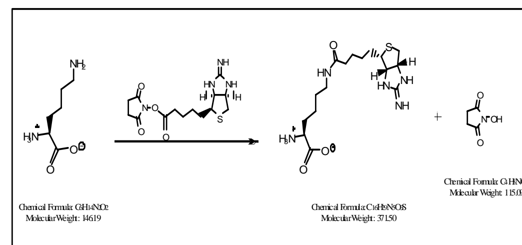
Plasma membrane proteins, such as G protein-coupled and tyrosine kinase receptors represent important members of the "druggable" proteome. Defining the complement of plasma membrane proteins displayed on living cells is crucial to discovering diagnostic disease biomarkers, therapeutic agents and drug receptor candidates. A modified affinity tagging procedure was employed to selectively label cell surface proteins. A high yield method relying upon detergents to sequentially extract proteins from small numbers of starting material was then employed to fractionate eukaryotic cells on a physiochemical basis into four distinct protein fractions: cytosolic, plasma membrane plus organelle, nuclear, and cytoskeletal. With adherent cells, the sequential extraction is performed directly in the tissue culture dish without removing the cells. At each step of the extraction procedure the insoluble cellular fractions remain attached to the plate, until the appropriate extraction reagent is applied. For suspension-grown cells, extraction starts with gentle sedimentation and washing of the cells. The stepwise extraction delivers all four protein fractions from a single specimen. Extracellularly-exposed plasma membrane proteins from the fractions are then selectively visualized using a fluorophore-labeled reporter conjugate after polyacrylamide gel electrophoresis and subsequently protein bands of interest are trypsinized, followed by matrix assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF-MS)-based peptide mass fingerprinting or tandem mass spectrometry-based peptide sequencing. Overall, combining the labeling procedure with the fractionation procedure improves detection of low abundance plasma membrane proteins and allows monitoring of changes in their association with the underlying cytoskeleton. The outlined labeling and detection workflow should be suitable for the characterization of membrane proteins by a variety of standard gel-based proteomics methods.

Diagram of the 2-iminobiotin at low and high pH values



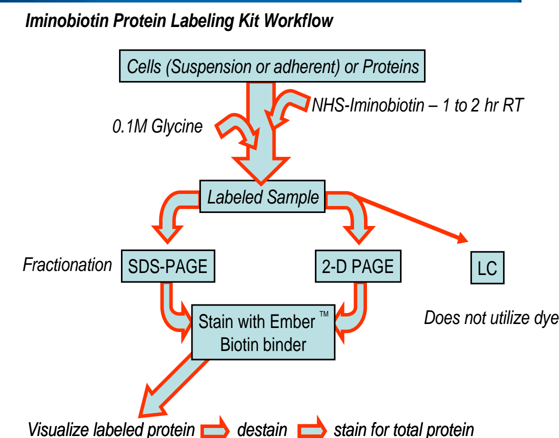
2-iminobiotin forms a high affinity complex with avidin (Ka = 10^8 M(-1). Binding is pH-dependent— at high pH values, the free base form of 2-iminobiotin retains the high affinity, selective avidin binding property of biotin, whereas at acidic pH values, the salt form of the biotin analog interacts poorly with avidin. This makes purification of compounds conjugated to avidin or iminobiotin possible under mild conditions, by affinity chromatography. At low pH the avidin-iminobiotin complex is converted to the avidin-biotin complex in the presence of exogenously added biotin.

Diagram of the reaction of 2-iminobiotin with lysine



NHS-iminobiotin reacts with α-amino groups at the N-termini of proteins/peptides and with ε-amino groups of lysine residues to form stable amide linkages that are fairly resistant to hydrolysis. NHS-iminobiotin labeling is compatible with conventional SDS-PAGE and 2D gel electrophoresis.

Protein Labeling Workflow



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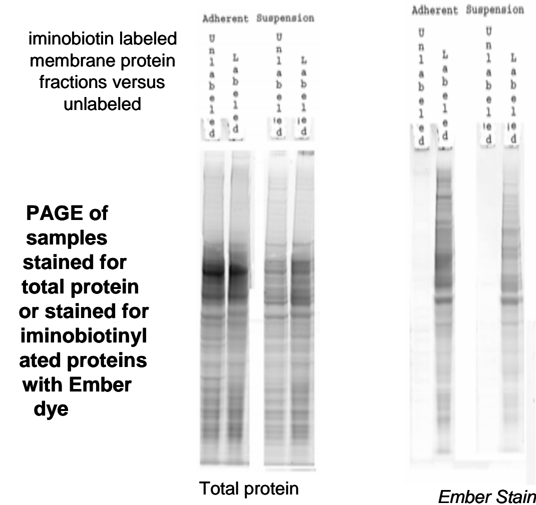
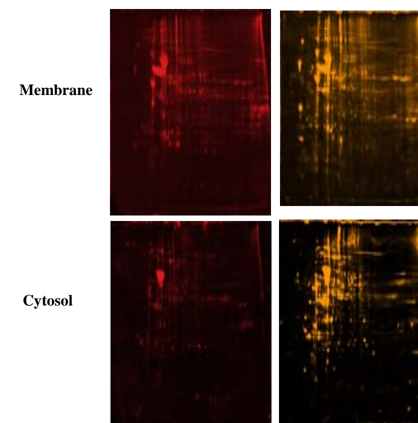
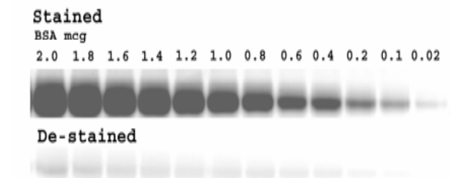
Membrane Labeling

Iminobiotin Protein Labeling: Subcellular Fractionation





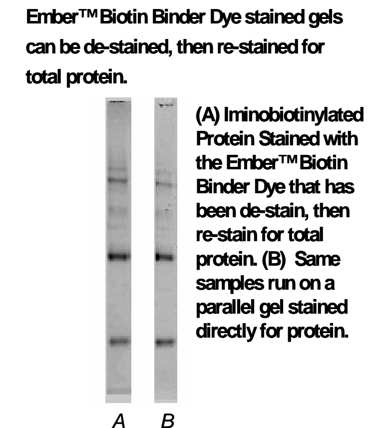
De-staining



PerkinElmer® Ember™ Biotin Binder Dye de-staining. SDS-PAGE gel with decreasing amounts of iminobiotinylated BSA after stained with the dye. Same gel de-stained and re-imaged (bottom) using same imager settings.

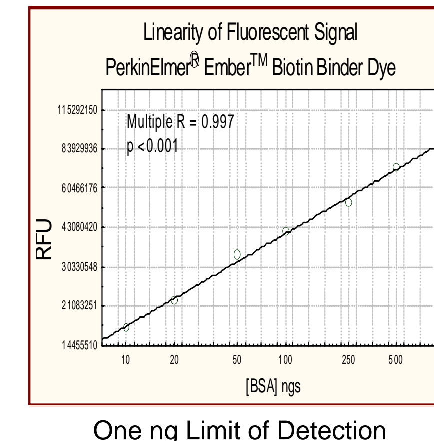
Re-staining

Ember™ Biotin Binder Dye stained gels can be de-stained, then re-stained for total protein.



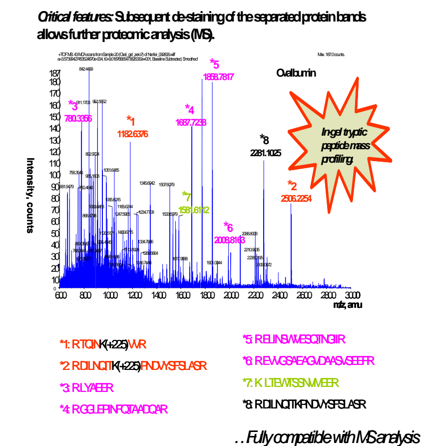
(A) Iminobiotinylated Protein Stained with the Ember™ Biotin Binder Dye that has been de-stain, then re-stain for total protein. (B) Same samples run on a parallel gel stained directly for protein.

Linear Dynamic Range



One ng Limit of Detection

MS Compatibility



Conclusions

- ➢Tool for reversible in-gel biotin detection and plasma membrane protein enrichment.
- ➢Labeling is selective for cell surface proteins.
- ➢Compatible with a wide-range of enrichment strategies for plasma membrane proteins (conventional centrifugation methods, differential detergent extraction and affinity purification using streptavidin- or avidin-based capture matrices).
- ➢Suitable for SDS-PAGE or 2-D gel electrophoresis analysis of any iminobiotinylated molecule, including plasma membrane proteins.
- ➢Iminobiotinylation does not alter protein isoelectric point.
- ➢Labeled proteins are easily detected after PAGE by staining with the red-fluorescent PerkinElmer® Ember™ Biotin Binder Dye. No electroblotting is required.
- ➢Subsequent de-staining of the separated protein bands allows restaining for total protein detection or further proteomic analysis (MS).

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