Capture of Tagged Proteins and Complexes with Enhanced Visibility Affinity Resins

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Abstract
It is often desirable to isolate recombinant proteins from biochemical preparations by small-scale affinity capture techniques, such as immunoprecipitation (IP). We developed a line of affinity capture resins with enhanced visibility (EZview Affinity Gel), which facilitate manipulation, such as washing and retrieval of supernatant from small affinity resin pellets, while reducing the possibility of sample loss.

In this work we demonstrate the utility of these enhanced visibility resins to capture epitope-tagged recombinant proteins and biochemically-tagged proteins for protein-protein interaction studies. We show that these enhanced visibility resins are equivalent to standard, non-colored resins in terms of target protein capture and non-specific background, but with improved visibility, enabling the rapid analysis and identification of bound epitope-tagged proteins.

Introduction

Goal – To test novel affinity resins with enhanced visibility for use in immunoprecipitation (IP). Ex 1 to study protein-protein interactions.

Approach
• Test direct capture of an epitope-tagged target protein with an antibody
• Test direct capture of a biotinylated protein with a streptavidin resin
• Test utility for protein-protein interaction studies.

Direct Affinity Capture Resins
Specific antibodies can be covalently attached to a solid matrix, such as agarose, by various chemical methods to form affinity resins that directly capture the antigen target proteins. This coupling method can improve the efficiency of protein capture by immobilizing antibody molecules bound in undefined orientations or inactive antibodies.

Antibodies and other proteins can be easily modified by the attachment of biotin at sites specific to antibody. Subsequently, biotinylated antibodies can be rapidly and tightly bound to streptavidin agarose to form specific affinity resins. This approach can be improved by using biotinylated antibodies with identified epitope tags. We and others have used this method to generate a variety of specific affinity resins.

Ezview Affinity Gel Performance
We made enhanced visibility antibody affinity gel resins and streptavidin affinity gel resin and compared them to analogous standard affinity gels for the capture of target proteins.

Affinity Capture of an Epitope-Tagged Protein
Affinity Capture of a Biotinylated Protein
EZview Standard and EZview Red ANTI-FLAG Affinity Gel were compared for immunoprecipitation of a FLAG epitope-tagged protein from a cell lysate (Fig. 3). The enhanced visibility (EZview) gel affinity gel performed similarly to the standard affinity gel.

Streptavidin Affinity Gel
We compared standard and EZview Red ANTI-FLAG Affinity Gel resins for affinity capture of a biotinylated target protein from a cell lysate (Fig. 4). The enhanced visibility (EZview) gel affinity gel performed similarly to the standard affinity gel.

Background

We developed highly visible colored resins, EZview Red Affinity Gel (Fig. 1), to improve the visibility of protein complexes formed on affinity resins, enabling rapid detection, analysis and identification of bound epitope-tagged proteins.

EZview Affinity Gels
EZview Standard and EZview Red were compared for affinity capture of a biotinylated protein from a cell lysate (Fig. 4).

Streptavidin Affinity Gel
EZview Standard and EZview Red were compared for affinity capture of a biotinylated protein from a cell lysate (Fig. 4).

Identification of Interacting Proteins
Matrix Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-MS) has become an important method for identifying proteins by signature masses of derived tryptic digest peptides.

Isolation of Interacting Proteins
We immunoprecipitated an expressed FLAG-tagged target protein from transfected COS-7 cell lysates using biotinylated ANTI-FLAG M2 monoclonal antibody and EZview Red Streptavidin Affinity Gel. The proteins were subjected to SDS-PAGE analysis (Fig. 4) and subjected to trypsin reduction and alkylation, followed by in-gel tryptic digestion.

Discussion

Affinity resins with enhanced visibility are easy to see and can improve quantitative recovery of the resin and target molecules for more reproducible results.

Conclusion

We would like to thank Stephanie Iliadis, Ken Heumann, Rob McGhie and the Sigma-Aldrich Biotechnology Protein R&D group for discussions and suggestions during this work. Also, we thank the Sigma-Aldrich Biotechnology Program group for support of Patty Lindsay, Max Huang and Tom Buttsik, who also participated in the development of the EZview Red Affinity Gel.}

References

Materials

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*Patent Pending