Affinity Capture Resins With Enhanced Visibility

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Abstract
Small-scale affinity capture techniques, such as immunoprecipitation (IP), are commonly used to isolate proteins from biochemical preparations. Procedures typically involve capturing the target protein from the reaction mixture by binding antibodies to an agarose resin, followed by repeated centrifugation and washing steps.

The inherent low visibility of standard agarose resin pellets makes washes and removal of supernatants difficult and prone to errors due to accidental removal of the poorly visible affinity matrix. We developed EZview affinity resins to facilitate supernatant removal without sample loss. We demonstrate that enhanced visibility protein A and protein G resins efficiently capture rabbit and mouse IgG antibodies. Using antibodies to the FLAG-tag or phosphotyrosine for in vitro COS-7 cell transfections, we show these resins outperform standard protein A or protein G resins in terms of target protein capture and non-specific background, but with improved handling characteristics.

Introduction

Goal - To develop affinity resins with enhanced visibility for use in immunoprecipitation (IP) and other molecular pull-down experiments (Fig. 1).

Desired Attributes
- More visible than standard, non-colored affinity beads.
- Low non-specific protein binding.
- Compatible with popular affinity capture techniques.
- Approach
- Conjugate dyes to agarose beads and screen for low protein binding.
- Make colored affinity beads.
- Test in affinity capture applications.

Background

Day ligand affinity chromatography has been a common tool used by researchers in protein purification schemes. An organic dye molecule, covalently attached to a solid matrix, is used as a ligand, commonly a polyclonal or monoclonal antibody, to enrich the molecules of interest from a mixture. Such dye-containing resins tend to bind large amounts of proteins with varying affinities. In contrast, to have a highly visible colored resin for IP applications, we developed EZview Red Affinity Gels, agarose resins with a covalently linked dye that binds only trace amounts of non-specific proteins (Fig. 2).

Affinity Resins for IP

EZView™ Red Protein A and Protein G Affinity Gels

Protein A and protein G are bacterial cell wall proteins that bind IgG antibodies. When covalently attached to a solid matrix, such as agarose, these proteins can be used to capture and purify antigen-antibody complexes from biochemical solutions. We compared standard and EZview red protein A and protein G agarose affinity gels for IgG capture (Fig. 3 and Fig. 4) and for immunoprecipitation of antigen proteins (Fig. 5 and Fig. 6).

Phosphotyrosine Protein IPs

Fig. 2. Enhanced visibility of EZView™ Red Affinity Gel (patent pending).

Fig. 3. EZView Red Protein A and Protein G Affinity Gels have similar performance to standard protein A and protein G agarose for immunoprecipitation (IP) applications.

Fig. 4. EZView Red Protein A and Protein G Affinity Gels have similar performance to standard protein A and protein G agarose for immunoprecipitation (IP) applications.

Fig. 5. Tag specific EZView Red Affinity Gels perform similar to standard affinity gels for direct capture of tagless tagged proteins. Target protein (HAT™-tagged bacterial alkaline phosphatase, HAT-BAP) was either spiked (lanes 1, 3) or not spiked (lanes 2, 4) into COS-7 cell lysates (10⁷ cells in 1 ml RIPA buffer). Lanes 1 and 2 were probed with EZView Red Protein A Affinity Gel. The gel was stained with colloidal blue stain (EZBlue™ Gel Staining Reagent). Lanes 3 and 4 were probed with HAT™-tagged commercially available antibody and developed with BCIP/NBT substrate.

Discussion

1) Resins were made with enhanced visibility by conjugating low protein binding dyes to agarose.
2) EZView Red Protein A and Protein G affinity gels were developed and demonstrated to be functionally equivalent to standard protein A and protein G agarose affinity gels in IP applications.
3) EZView Red Anti-FLAG M2 and His Select affinity gels were developed and demonstrated to be functionally equivalent to standard Protein A and Protein G agarose affinity gels, but with improved handling characteristics.

Conclusion

Affinity resins with enhanced visibility that bind low amounts of non-specific protein have advantages over standard affinity resins:

1) More readily visible.
2) More rapid manipulation possible.
3) Improved quantitative recovery of the target and target molecules for more reproducible results.

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References