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 a complex mixture with a specific antibody. The antibody-antigen complexes are isolated by capture with protein A or protein G covalently attached 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 error, due to accidental removal of the poorly visible affinity matrix. We developed high visibility protein A and protein G 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 IP from COS-7 cell lysates, 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 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.

Immunoprecipitation

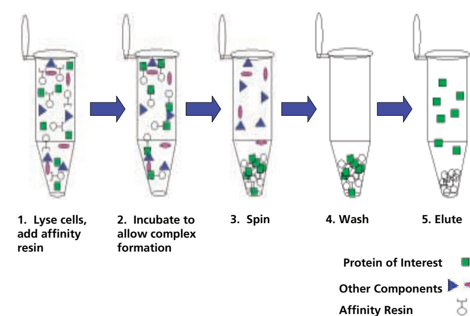

Fig. 1. Schematic representation of affinity based molecular pull-down methods, such as immunoprecipitation.

Background

Dye 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 such as beaded agarose, can mimic physiological ligands for proteins. This enables proteins with affinity for the dye molecule to be easily separated from a heterogeneous biological preparation. Such dye containing resins tend to bind large amounts of proteins with varying affinities.

In contrast, to make a highly visible colored resin for IP applications, we developed EZview Red Affinity Gels, agarose resins with a covalently attached dye that binds only trace amounts of non-specific proteins **(Fig. 2)**.

Enhanced Visibility Affinity Gel

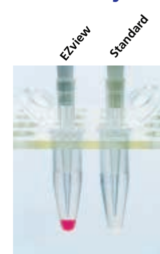

Fig. 2. Enhanced visibility of EZview™ Red Affinity Gel (patent pending).

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 antibody molecules. When covalently attached to a solid matrix, such as cross-linked 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 4)** and for immunoprecipitation of protein antigens **(Fig. 5 and Fig. 6)**.

mAb Capture: Standard vs EZview

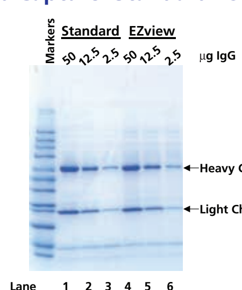

Fig. 3. EZview Red Protein A Affinity Gel and standard protein A agarose recover a similar amount of IgG. Different amounts of ANTI-FLAG® M2 monoclonal antibody were spiked into COS-7 lysates (10^7 cells in 1 ml RIPA buffer). The antibody was captured using standard protein A agarose (**Standard**, lanes 1-3) or EZview Red Protein A Affinity Gel (**EZview**, lanes 4-6). After washing the affinity beads, the bound proteins were eluted and subjected to SDS-PAGE. The gel was stained with colloidal blue stain (EZBlue™ Gel Staining Reagent).

mAb Capture: Protein A vs G

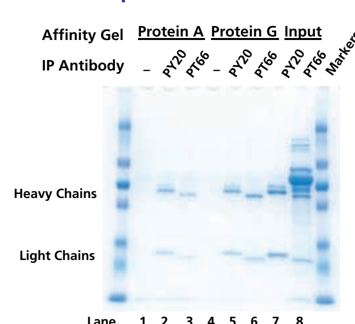

Fig. 4. EZview Red Protein G Affinity Gel recovers more mAb (IgG1) than EZview Red Protein A Affinity Gel. Two different mouse monoclonal antibodies (**PY20** and **PT66**; both IgG1 subclass) were spiked, or not spiked (-), into COS-7 lysates and captured with either EZview Red Protein A (**Protein A**) or EZview Red Protein G (**Protein G**) Affinity Gel. After washing the affinity beads, the bound proteins were eluted and subjected to SDS-PAGE. The gel was stained with EZBlue Gel Staining Reagent.

IP with EZview Affinity Gels

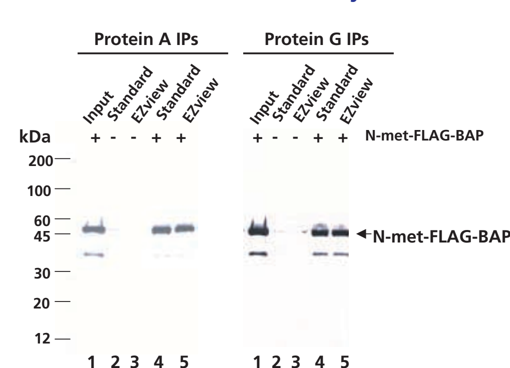

Fig. 5. EZview Red Protein A and Protein G Affinity Gels have similar performance to standard protein A and protein G agarose for immunoprecipitation (IP). Target proteins were either spiked (+), or not spiked (-), into COS-7 cell lysates and captured using ANTI-FLAG M2 monoclonal antibody and either **standard** and **EZview Red Protein A Affinity Gel (left panel)** or **standard** and **EZview Red Protein G Affinity Gel (right panel)**. Western blots of SDS-PAGE gels are shown. The blots were probed with ANTI-FLAG-M2 monoclonal antibody conjugated to alkaline phosphatase and developed with BCIP/NBT substrate.

Phosphotyrosine Protein IPs

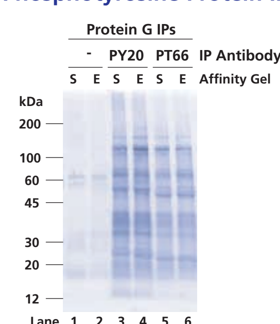

Fig. 6. EZview Red Protein G Affinity Gel has similar performance to standard protein G agarose for IP with anti-phosphotyrosine (pTyr) specific antibodies. Endogenous phosphotyrosine-containing proteins were captured from COS-7 lysates with two different anti-pTyr monoclonal antibodies, **PY20** and **PT66**, or without antibody (-), and either **standard (S)** or **EZview Red Protein G Affinity Gel (E)**. A Western blot of an SDS-PAGE gel is shown. The blot was probed with PT66 conjugated with biotin followed by streptavidin-peroxidase polymer and developed with TMB substrate.

Affinity Resins for Capture of Tagged Proteins

Many affinity capture resins have an epitope tag-specific antibody, or other capture ligand, covalently attached to the resin.

ANTI-FLAG® M2 agarose affinity gel has the M2 monoclonal antibody (specific for the FLAG peptide) attached to beaded agarose. EZview Red ANTI-FLAG M2 agarose was shown to perform equivalently to standard, non-colored ANTI-FLAG M2 agarose for direct affinity capture of a FLAG-tagged protein **(Fig. 7, left panel)**.

HIS-Select™ affinity gel has a proprietary tetradentate nickel chelate attached to beaded agarose. EZview Red HIS-Select affinity gel was shown to perform equivalently to standard, non-colored HIS-Select affinity gel for direct affinity capture of a histidine-tagged protein **(Fig. 7, right panel)**.

Tag Specific EZview Affinity Gels

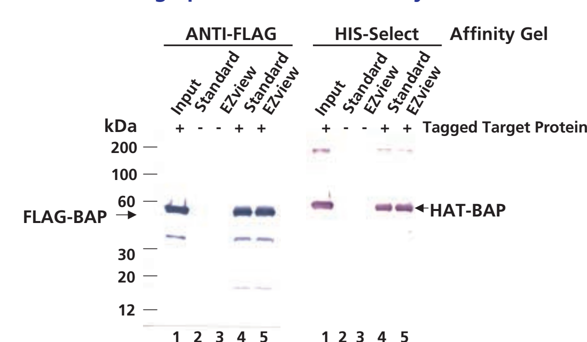

Fig. 7. Tag specific EZview Red Affinity Gels perform similar to standard affinity gels for direct capture of epitope tagged proteins. Tagged target proteins (FLAG-tagged bacterial alkaline phosphatase, FLAG-BAP, or HAT™-tagged BAP, HAT-BAP) were either spiked (+), or not spiked (-), into COS-7 cell lysates and captured using either **standard** and **EZview Red ANTI-FLAG Affinity Gel (left panel)** or **standard** and **EZview Red HIS-Select HC Nickel Affinity Gel (right panel)**. Western blots of SDS-PAGE gels are shown. The blots were probed with ANTI-FLAG-M2 monoclonal antibody conjugated to alkaline phosphatase (left panel) or anti-HAT™ antibody with alkaline phosphatase conjugated secondary antibody (right panel) and developed with BCIP/NBT substrate. HAT is a trademark of Clontech, Palo Alto, CA.

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 ANTI-FLAG M2 and HIS-Select affinity gels for direct affinity capture of tagged proteins.

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 resin and target molecules for more reproducible results.

Acknowledgements

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Related Sigma Products

Product Name	Product Number
EZview™ Red Protein A Affinity Gel*	**P 6486**
EZview™ Red Protein G Affinity Gel*	**E 3403**
EZview™ Red ANTI-FLAG® M2 Agarose Affinity Gel*	**F 2426**
EZview™ Red HIS-Select™ HC Nickel Affinity Gel*	**E 3528**
Protein A Agarose	P 2545
Protein G Agarose	P 7700
ANTI-FLAG® M2 Affinity Gel	A 2220
HIS-Select™ HC Nickel Affinity Gel	P 6611
NBT/BCIP Alkaline Phosphatase Substrate	B 5655
EZBlue™ Gel Staining Reagent	G 1041
ANTI-FLAG® M2 mAb-AP Conjugate	A 9469
Monoclonal Anti-Phosphotyrosine, Clone PY-20	P 4110
Monoclonal Anti-Phosphotyrosine, Clone PT-66	P 3300
Monoclonal Anti-Phosphotyrosine, Biotin Conjugate, Clone PT-66	B 1531
Streptavidin-Peroxidase Polymer, Ultrasensitive	S 2438
3,3',5,5' Tetramethylbenzidine (TMB) Liquid Substrate	T 0565

***Patent Pending**