



How to Capture at Least 2x More Antigen in an Immunoprecipitation

Introduction

The binding interaction between biotin and streptavidin is one of the strongest non-covalent bonds known in nature. With a dissociation constant (K_d) of approximately 10^{-14} M, the streptavidin-biotin complex is essentially non-dissociable, even under standard denaturing conditions. This extraordinary affinity has led to the prolific use of the biotin-streptavidin couple in biotechnology and nanotechnology, as it allows rapid binding kinetics with relatively low non-specific binding. Applications are many, and include immunoprecipitations, Western, Northern, and Southern blotting, enzyme linked immunosorbent assay, immunohistochemistry, and affinity purification, among others.

The Solulink Advantage

Solulink's Streptavidin Agarose Ultra Performance™ was specifically developed with several key parameters in mind — high biotin binding capacity and low non-specific binding, on an agarose support which would support high flow rates with consistent permeation. These qualities make it an ideal media for applications such as immunoprecipitation (IP), co-immunoprecipitation (co-IP), chromatin immunoprecipitation (ChIP), affinity purification, cell capture, etc. Solulink utilizes a recombinant streptavidin which is of high purity, possessing much lower non-specific binding than avidin due to its lower isoelectric point and lack of glycosylation. This streptavidin is immobilized via Solulink's proprietary linking technology, resulting in highly stable immobilizations with minimal protein leaching. Moreover, unlike other conjugation chemistries such as cyanogen bromide, Solulink's conjugation process leaves the agarose with no net charge which could otherwise interact with biomolecules to cause unwanted binding. Our core agarose is specially formulated to be of unparalleled uniformity, with a relatively small mean diameter of 35µm. This, combined with its high level of crosslinking, creates a resin which is tolerant of high flow rates or centrifugal forces often experienced in high-throughput screening applications and immunoprecipitations.

Example Application

To demonstrate the superior results achievable with Solulink's Streptavidin Agarose Ultra Performance™, an immunoprecipitation of β -actin from whole cell lysate was performed with subsequent detection via Western blot. Mouse monoclonal anti- β -actin (100µg) was biotinylated with Solulink's quantifiable biotinylation reagent [ChromaLink™ Biotin](#). A simple UV spectrophotometric scan of the biotinylated antibody reveals both the biotin Degree of Labeling (DOL) and protein concentration (Figure 1).

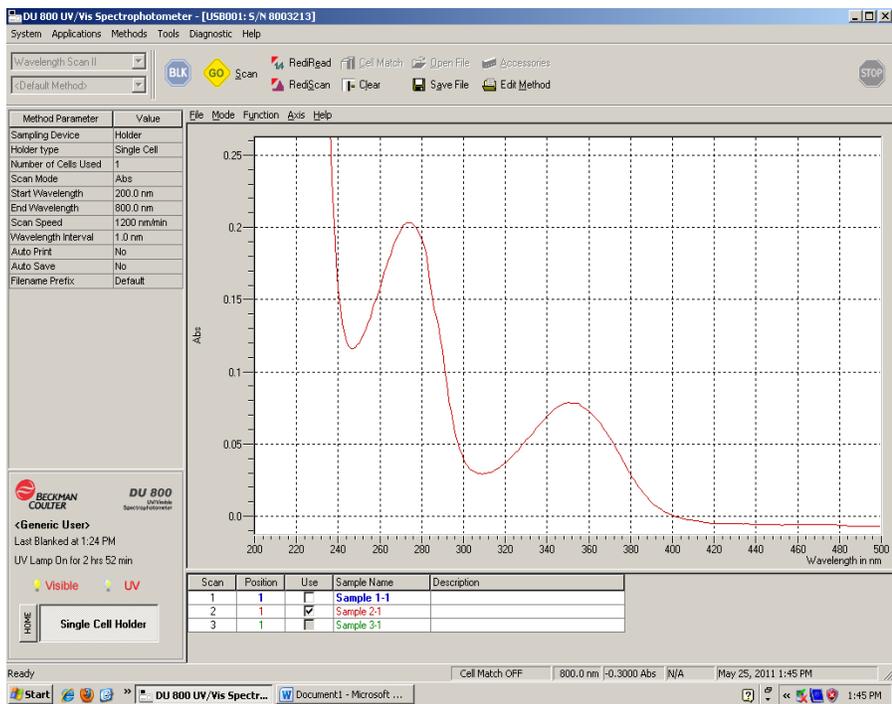


Figure 1. Simple measurement of antibody A280 and A354 yields both antibody concentration and number of incorporated biotins per antibody.

2µg of anti-β-actin (DOL = 3.1 biotins per antibody) was incubated with 5µL of settled Streptavidin Agarose Ultra Performance™ (or competitor resin) for 20 minutes to immobilize the antibody. Resin was subsequently pelleted at 1,000g for 2 minutes, washed with PBS, and 20µg of mouse Lewis lung carcinoma (LLC) lysate was added. After a 1 hour incubation, the resins were washed with three 0.5mL portions of PBST (PBS + 0.05% Tween 20). All resin slurry volumes were normalized to 20µL with PBS and β-actin was eluted from the immunoaffinity medium by heating at 85°C for 3 minutes in an equal volume of 2X reducing sample buffer.

A 4-12% gradient SDS-PAGE gel was run consisting of 10µL of eluted sample from each resin, along with 2.5µg of LLC lysate positive control. Proteins were subsequently transferred to a nitrocellulose membrane using a semi-dry transfer cell at 10V for 45 minutes. After blocking the membrane with 3% non-fat dry milk in PBST for 1 hour, the membrane was probed with 0.2 µg/mL mouse monoclonal anti-β-actin — HRP conjugate prepared using Solulink's [Rapid Direct Primary Antibody polyHRP Western Blot Kit](#). Bands were visualized using chemiluminescent HRP substrate with film exposure. Figure 2 compares the results obtained using Streptavidin Agarose Ultra Performance™ vs. competing streptavidin resins.

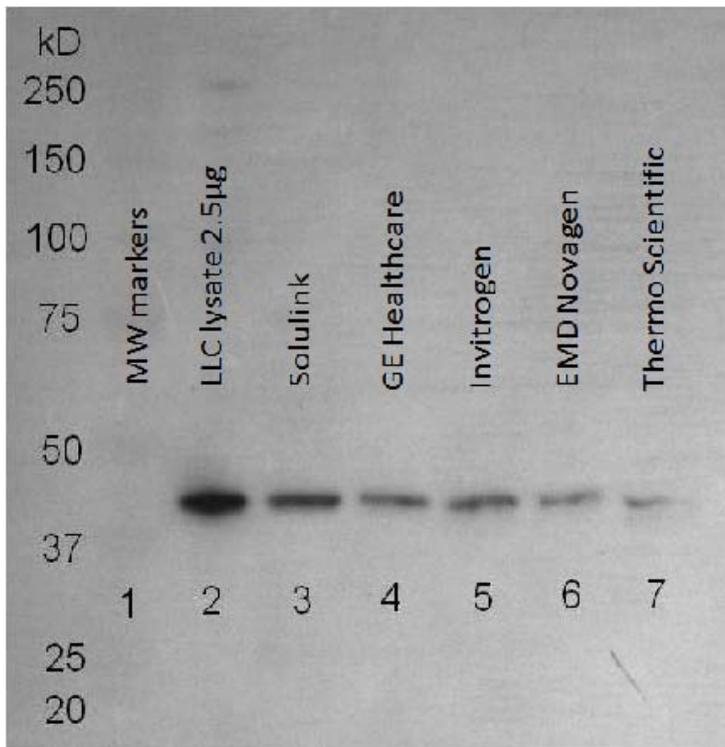


Figure 2. Western blot β -actin results -Solulink vs. competitors

Lane 1. MW marker
 Lane 2. 2.5 μ g LLC lysate control
 Lane 3. **Solulink Streptavidin Agarose Ultra Performance**
 Lane 4. GE Healthcare streptavidin resin
 Lane 5. Invitrogen streptavidin resin
 Lane 6. EMD Novagen streptavidin resin
 Lane 7. Thermo Scientific streptavidin resin

Conclusion

Based on immunoprecipitation of β -actin in this study, Solulink's Streptavidin Agarose Ultra Performance™ captured at least 2 times more antigen than competing streptavidin agarose products under identical conditions. This increased binding capacity, along with its rigid core beaded agarose, makes it the ideal medium for capture of biotinylated biomolecules at all scales. The medium may be used either in batch format or packed into chromatography columns to suit the scale and process at hand. The complementary biotinylation reagent, ChromaLink™ Biotin, makes biotinylation and subsequent determination of degree of labeling simple, without the need for HABA assays which are laborious, inaccurate, and consume large amounts of biotinylated sample. When used with the Rapid Direct Primary Antibody polyHRP Western Blot Kit, only the band of interest is detected. Interfering contamination from antibody light and heavy chains is no longer an issue. Also, the poly-HRP nature of the conjugate makes the detection reagent more sensitive than primary/secondary antibody detection techniques. From biotinylation with ChromaLink™ Biotin, to capture with Solulink's high-binding Streptavidin Agarose Ultra Performance™ and detection with the Rapid Direct Primary Antibody polyHRP Western Blot Kit, Solulink offers a distinct advantage to each step of the immunoprecipitation workflow.

Related Products:

Cat. No.	Description	Size	Price
N-1000-005	Streptavidin Agarose Ultra Performance	5 mL *also available in 2 mL and 10 mL	\$295.00
B-1007-105	ChromaLink™ Biotin Labeling Reagent Water Soluble	5 x 1.0 mg	\$225.00
A-9401-001	Rapid Direct 1° Antibody polyHRP Western Blot Kit	Kit	\$250.00