Simple, rapid streptavidin-based enrichment using Capturem technology

- 5- to 15-minute enrichment protocol of biotinylated compounds, including antibodies, proteins, and nucleic acids
- Available in 96-well plates for high-throughput applications, compatible with centrifugation or vacuum and positive pressure automated systems
- High well-to-well reproducibility ensures consistent results for your assays

Introduction

Streptavidin-based capture plays an important role in protein chemistry and antibody discovery workflows. The high affinity of streptavidin for biotin enables biotinylated moieties (proteins, peptides, antibodies, DNA, oligonucleotides, etc.) to be captured and immobilized on the Streptavidin surface. The immobilized molecule is then used to specifically capture a target protein (antibody or antigen) from complex matrices, allowing the purified target to be used for very precise downstream assays. Capturem products are built on a revolutionary high-capacity membrane technology that enables extremely fast purifications—in less than 15 minutes—without sacrificing yield or purity. Capturem Streptavidin Miniprep Columns and the Capturem Streptavidin 96-Well Plate use streptavidin-functionalized membranes to enable rapid capture of your biotinylated reagents. The loading capacities of the Capturem Streptavidin Miniprep Columns and Capturem Streptavidin 96-well Plates are similar, as shown below.

Loading capacities and times for available Capturem Streptavidin formats.

	Biotin-antibody	Biotin-BSA	Biotin-ssDNA	Free biotin	Purification time
Capturem Streptavidin miniprep columns and 96-well plates	20–40 µg	>15 µg	1,000–1,500 ng	>4,000 pmol	15 minutes

Results

Successive antibody capture

One major application for streptavidin-based pull-down experiments is the targeted enrichment of monoclonal antibodies (mAb) and proteins from complex mixtures. Here we demonstrate the use of Capturem Streptavidin to enrich an anti-rabbit IgG using a successive capture protocol that takes less than 15 minutes from start to finish. This is significantly faster than traditional methods that require incubation steps that can take up to 90 minutes.

Figure 1. Workflow for successive antibody capture.

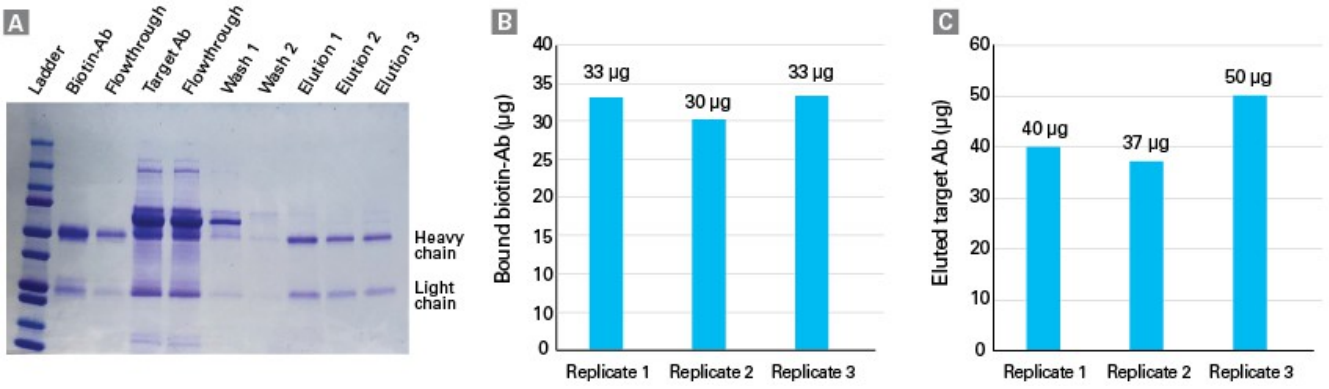

Figure 2. Successive capture of antibodies in triplicate using Capturem Streptavidin. Panels A–C. First, 48 µg of biotinylated rabbit IgG in 200 µl Binding Buffer was passed through an equilibrated Capturem Streptavidin spin column, and 32.0 ± 1.4 µg (**Panel B**) was successfully immobilized on the membrane. Following a single wash step, a sample containing the spiked-in target antibody (~100 µg of anti-rabbit IgG from goat) in hybridoma medium with 20% mouse serum was diluted with Binding Buffer and applied to the Capturem Streptavidin column. After two successive washing steps with Binding Buffer and then PBS, the enriched target antibody was eluted from the biotinylated capture antibody in 1.0 M glycine in three steps to yield 42 ± 5 µg (**Panel C**) of highly pure target antibody.

Highly reproducible capture for high-throughput applications

Maintaining consistent performance is critical for many purification applications involving downstream quantitative analysis. We measured well-to-well reproducibility of Capturem Streptavidin 96-Well Plates using both biotinylated BSA and biotinylated oligos.

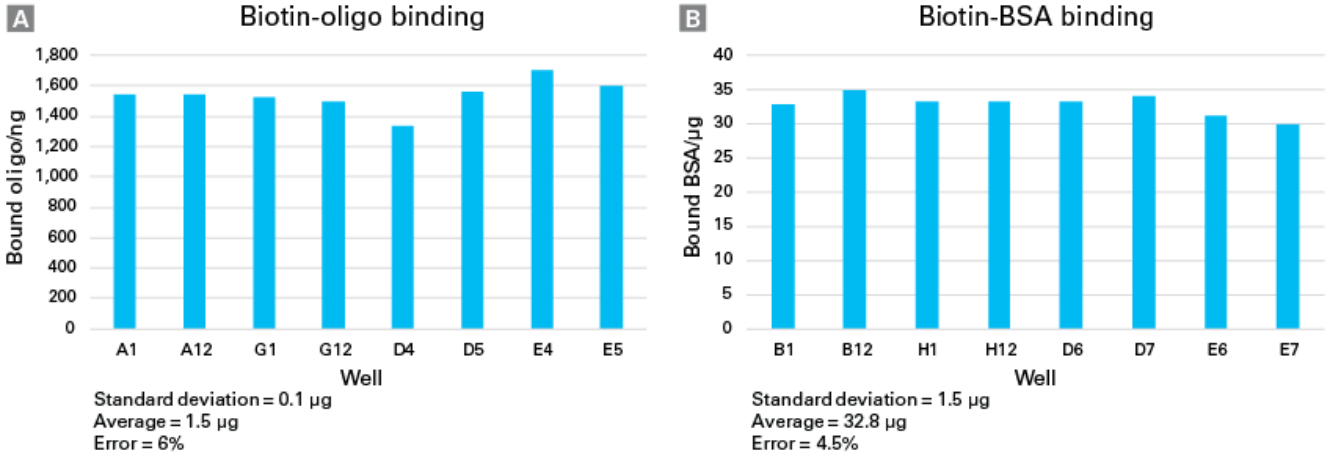

Figure 3. Highly reproducible capture in Capturem Streptavidin 96-Well Plates. Eight technical replicates were loaded with either biotinylated oligonucleotide (**Panel A**) or biotinylated BSA (**Panel B**) and captured using centrifugation. Bound molecules were quantified by measuring the O.D. of samples before and after loading through the column. Standard protocols with these 96-well plates can be performed in under 15 minutes.

Conclusion

Capturem Streptavidin enables rapid immobilization (or binding) of biotinylated compounds. Successive capture protocols can be performed using Capturem Streptavidin that dramatically shortens the hands-on time as compared to traditional methods, and these products are also compatible with traditional preincubation enrichment approaches. Capturem Streptavidin is available in both mini spin column and 96-well plate formats for both smaller experiments and high-throughput applications.

Methods

Successive antibody capture

Spin columns were first equilibrated by adding 800 µl Equilibration Buffer to the column followed by centrifugation at 500*g* for 1 min. Next, spin columns were loaded with 200 µl of biotinylated rabbit IgG at 0.24 mg/ml, then centrifuged at 500*g* for 1 min at room temperature to immobilize the biotinylated antibody. The columns were then washed with 400 µl Wash Buffer. Subsequently, 200 µl of anti-rabbit IgG at a concentration of 0.50 mg/ml was loaded onto the column and spun at 500*g* for 1 min. Two more wash steps were performed, first with 400 µl Wash Buffer and then with 400 µl of PBS. Finally, the captured secondary Ab was eluted using three successive elutions, each consisting of 90 µl of 1.0 M glycine. Eluted Ab was quantified using A_{280} measurements from a Nanodrop spectrophotometer. Bound biotinylated Ab was quantified by measuring the absorbance before and after loading.

Binding capacity and reproducibility experiments

Technical replicates of either biotinylated oligo (Biotin-G/AGCTTCATTTCCCGTAAATCCCTAAAGCT), biotinylated BSA, or biotinylated IgG were loaded into different wells of a 96-well plate for the reproducibility experiments (Figure 2) or into different spin columns for the capacity tests (Table). Absorbance measurements (A_{260} for the oligonucleotides, A_{280} for BSA and IgG) were made before and after loading to determine the amount of biomolecules bound to the membranes. For protein binding experiments 100 µg biotinylated BSA was diluted in 200 µl Binding Buffer and applied to each well while for oligonucleotide binding, 3.8 µg of oligo in 200 µl Binding Buffer were used.

Related Products

Cat. #	Product	Size	License	Quantity	Details
635734	Capturem™ Streptavidin 96-Well Plate	1 x 96-well plate		*	
	The Capturem Streptavidin 96-Well Plate is a single-use, disposable plate for simple, rapid enrichment of target proteins and antibodies that bind biotinylated protein. The plate binds more than 15 µg of biotinylated BSA control per well. This plate is suitable for enrichment of target proteins and antibodies, including those from animal serum, cell culture lysates (e.g., mammalian or bacterial cell lysates), and cell culture supernatants of hybridoma cell lines.				
	Documents / Components / Image Data				
635733	Capturem™ Streptavidin Miniprep Columns	20 Columns		*	

Add to Cart

Takara Bio USA, Inc.
United States/Canada: +1.800.662.2566 • Asia Pacific: +1.650.919.7300 • Europe: +33.(0)1.3904.6880 • Japan: +81.(0)77.565.6999