

## TECH NOTE

# Fast, Efficient, and Specific Immunoprecipitation and Co-Immunoprecipitation with Capturem Protein A Technology

Rapid Protein A column IP of PP2A B subunit >>

Compatibility with a variety of IP buffers >>

p53 and SV40 T Co-IP with Capturem Protein A minipreps >>

## Introduction

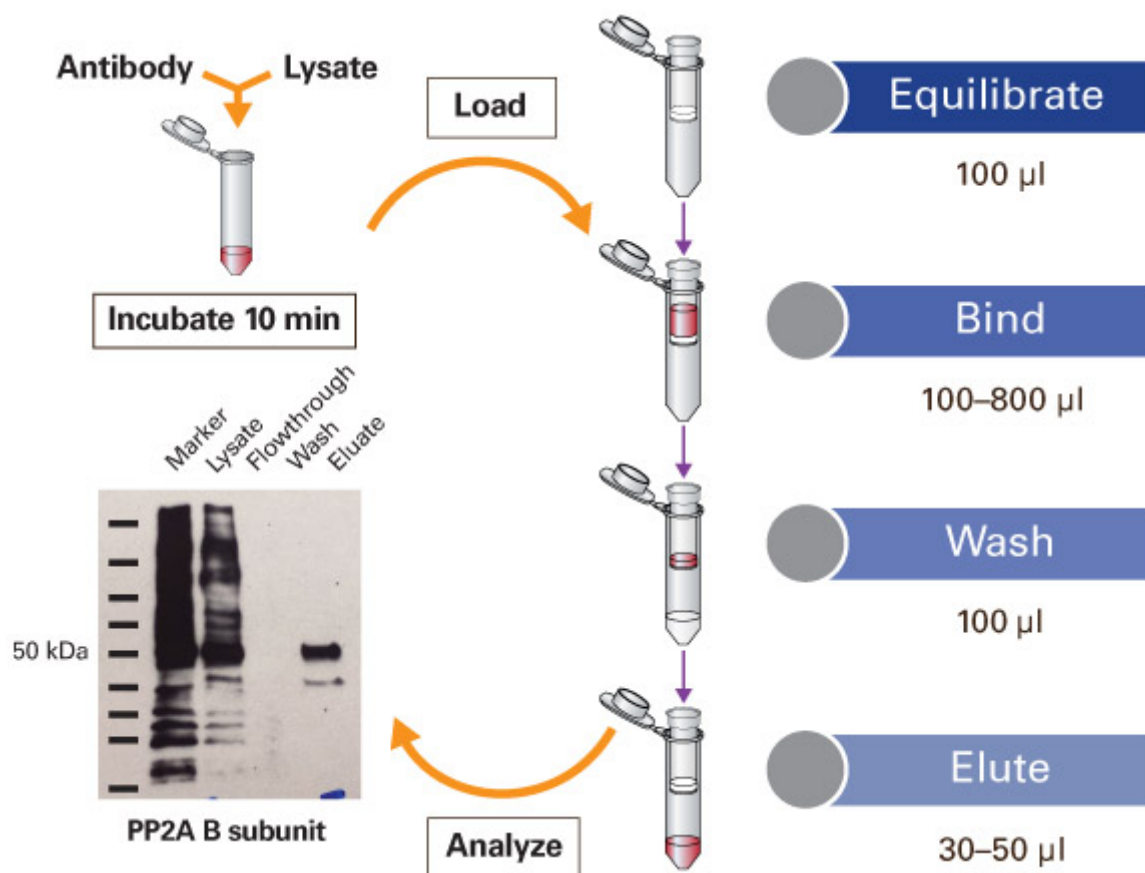
For nearly half a century, immunoprecipitation (IP) has been a cornerstone of cell and molecular biology research. In addition, it has proven to be a valuable screening technique for the development of novel biologics, such as antibody-based therapeutics. As academic and biopharmaceutical researchers look to accelerate the application of bench discoveries to the clinic, there is a constant need for faster and more efficient immunoprecipitation protocols.

Capturem Protein A columns combine the specific antibody-binding properties of Protein A with novel Capturem technology to provide high-capacity, membrane-based affinity purification. The antibody-binding properties also make these columns ideal for use in IP and co-immunoprecipitation (Co-IP) experiments. After optimization of the purification parameters, we combined the Capturem Protein A columns with an optimized IP buffer set to create the [Capturem IP & Co-IP Kit](#), which provides a complete, easy-to-use IP/Co-IP solution. The kit also has added utility for the purification and screening of antibody-based therapeutics, including antibody-drug conjugates. Furthermore, the benefits of this resin-free system—including speed, ease-of-use, flexibility, and yield—make it a powerful tool for both academic research institutions and biopharmaceutical organizations.

## Results

### Rapid Protein A column immunoprecipitation

The same no-waiting workflow that provides fast, high-quality antibody purification also enables immunoprecipitation experiments to be completed quickly and easily. Following a 10-minute incubation of the antibody-antigen complex, the kit requires only five minutes of hands-on time for efficient IP. In the experiment below, NIH3T3 lysates were incubated with 1 µg of a protein phosphatase type 2A (PP2A) B subunit antibody before being applied to equilibrated Capturem Protein A spin columns. After washing with 300 µl of wash buffer and eluting in 100 µl of elution buffer, the various fractions were resolved by gel electrophoresis, transferred onto polyvinylidene fluoride (PVDF) membranes, and probed with an anti-PP2A B antibody. The Capturem Protein A spin columns yielded excellent results, with a strong protein PP2A B signal in the eluate fraction. Note: This data was produced using buffer formulations that differ from those found in the final Capturem IP & Co-IP Kit (see Methods: Immunoprecipitation performed with Capturem Protein A columns).

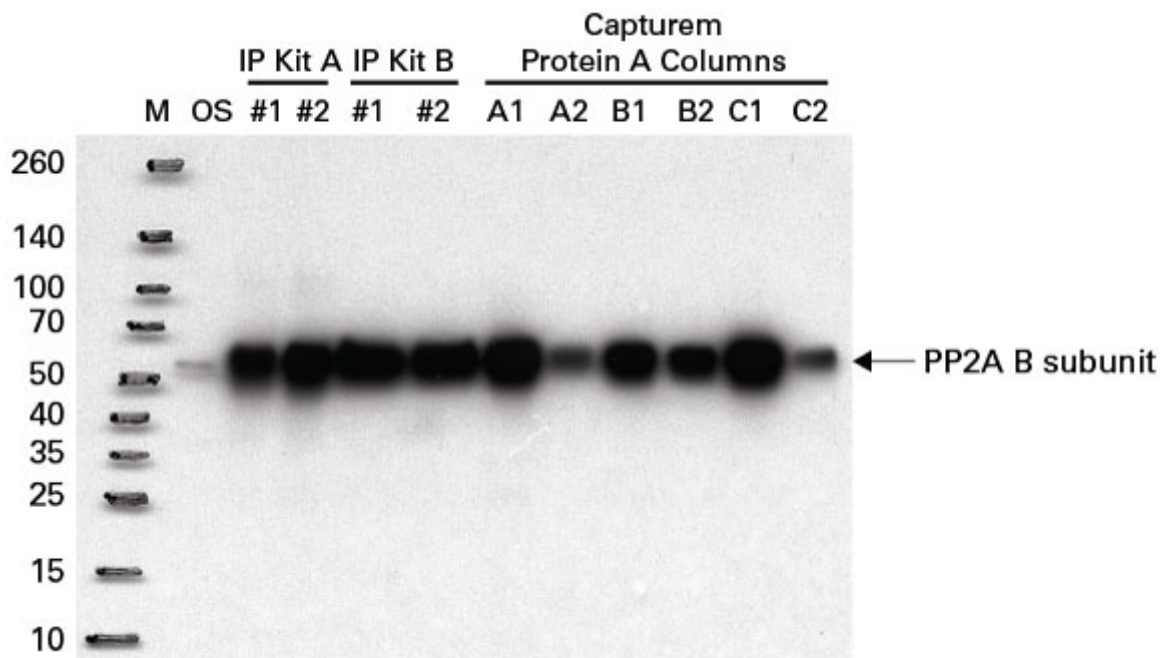


**Figure 1. Immunoprecipitation performed with Capturem Protein A columns.** NIH3T3 cell lysates were incubated with 1 µg PP2A B subunit antibody for 10 min. The antibody-lysate complex was applied to equilibrated Protein A spin columns. Following centrifugation and washing steps, elution was carried out in 100 µl of Elution buffer. Fractions were resolved by gel electrophoresis, transferred onto PVDF membranes, and probed with an anti-PP2A B antibody. The protein in the eluate fraction corresponds to the PP2A B subunit (52 kDa).

## Compatibility with a variety of immunoprecipitation buffers

Capturem Protein A products provide an excellent level of flexibility to accommodate a wide range of experimental conditions, including those performed with IP buffers from other kits. To test this aspect of performance, NIH3T3 cell lysates were incubated with a PP2A B subunit antibody in buffers provided with either IP Kit A, IP Kit B, or standalone lysis buffer C. After incubation, the antibody-lysate mixture was loaded onto Capturem Protein A spin columns that had been equilibrated with the corresponding IP buffer. Once centrifugation and wash steps were complete, elution was performed in 30 µl of elution buffer appropriate for each sample set (Figure 2; Capturem Protein A columns A1, B1, C1). Elution was repeated to ensure complete recovery of antibody complexes (Figure 2; Capturem Protein A columns A2, B2, C2). IP was also performed using the full IP kits A and B, per their respective protocols (Figure 2; IP Kit A, IP Kit B), starting with the same amount of lysate and antibody as used for Capturem columns.

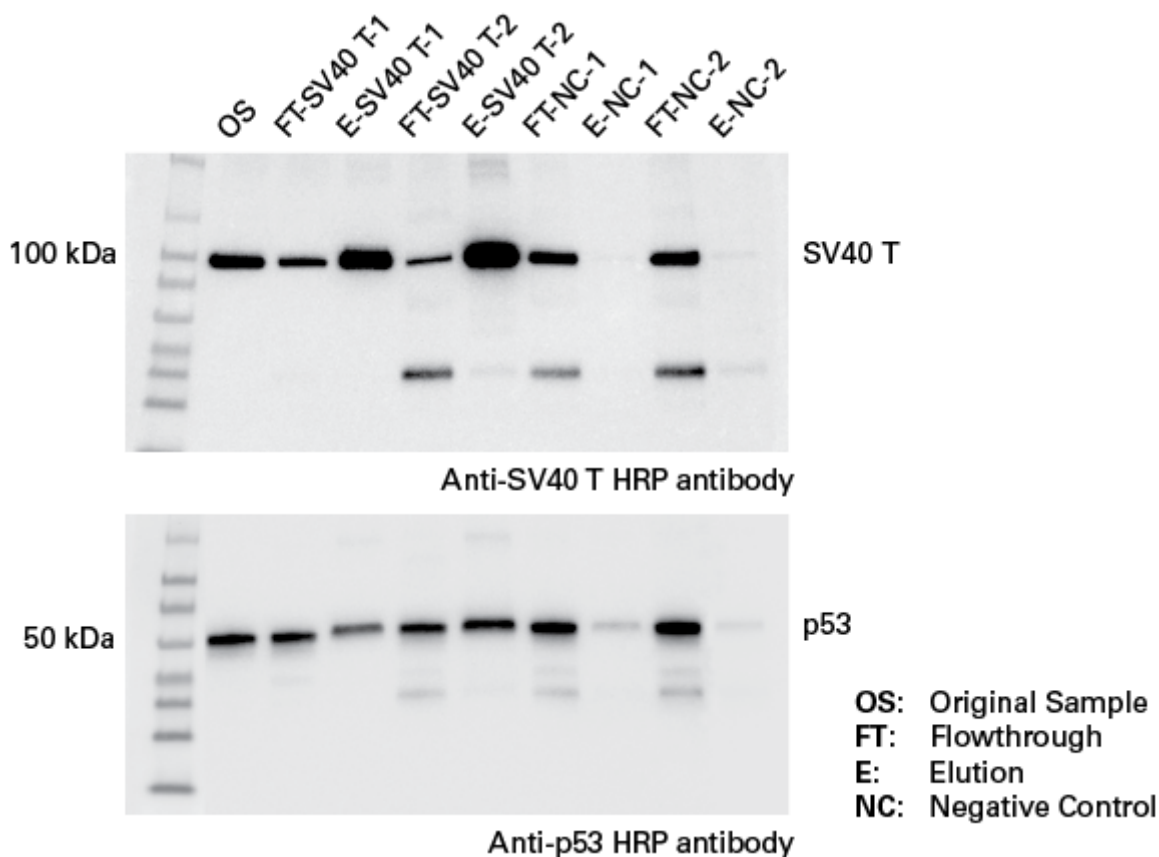
All samples were resolved on a gel, transferred to PVDF membranes, and probed for the PP2A B subunit using an appropriate antibody. The gel below shows the presence of the subunit in the original sample (OS), which is greatly enriched in the immunoprecipitated samples. Results from the Capturem Protein A columns showed strong PP2A B protein signal in the first elution, regardless of the IP buffer used, with the highest elution level for the Capturem samples eluted with a low pH glycine buffer (A1, C1). Note: This data was also produced using buffer formulations that differ from those found in the final Capturem IP & Co-IP Kit (see Methods: Immunoprecipitation performed with Capturem Protein A columns).



**Figure 2. Capturem Protein A columns are compatible with a variety of buffers.** NIH3T3 cell lysates (100  $\mu$ l, containing 130  $\mu$ g of total protein) were incubated with 0.6  $\mu$ g of protein phosphatase type 2A B subunit antibody in the IP buffers provided with either IP Kit A or B, or lysis buffer C, bringing the total volume up to 400  $\mu$ l. The antibody-lysate mixture was incubated at 4°C for 1 hr and was then loaded on Capturem Protein A columns that had been equilibrated with the corresponding IP buffer. Bound immunocomplexes were then eluted twice with 30  $\mu$ l of an appropriate elution buffer. IPs were also done using the full A or B IP kits. All elution samples were then resolved on a gel, transferred to PVDF membranes, and probed for the PP2A B subunit using an appropriate antibody. The presence of the PP2A B subunit in the original sample is shown in lane OS. Elution samples #1 and #2 are shown for those performed entirely with IP Kit A and IP Kit B. Elution samples #1 and #2 for Capturem Protein A columns are shown for those performed with buffers from IP Kit A (A1, A2), IP Kit B (B1, B2), and the standalone lysis buffer (C1, C2).

### Co-immunoprecipitation with the Capturem IP & Co-IP Kit

p53 is a 53-kDa nuclear phosphoprotein that functions as a tumor suppressor and is involved in inhibiting cell proliferation upon DNA damage. Wild-type p53 is known to form specific complexes with several viral oncogenes such as SV40 T antigen (SV40 T). Using 293T cells expressing both p53 and SV40 T, we demonstrated the ability to co-immunoprecipitate p53 and SV40 T at basal levels using Capturem Protein A columns. Anti-SV40 T antibody was added to lysates of these cells, and the mixture was incubated at room temperature for 20 minutes with end-over-end rotation. The IP was then performed following the protocol used for Figure 2, but using the buffer set provided in the Capturem IP & Co-IP Kit, which are optimized for both IP and Co-IP. The eluted samples were resolved on a gel and transferred to a PVDF membrane. The blots were then probed with mouse monoclonal antibodies against p53 and SV40 T. Capturem Protein A IP of SV40 T showed the presence of bands for both SV40 T (97 kDa) and p53 (53 kDa) in eluate fractions, confirming the strong interaction between these two proteins (Figure 3; E-SV40 T-1 and E-SV40 T-2).



**Figure 3. Co-immunoprecipitation performed with the Capturem IP & Co-IP Kit.** Using 293T cells expressing both p53 and SV40 T, we demonstrated the ability to Co-IP p53 and SV40 T at basal levels. To prepare the antibody-protein complexes for co-immunoprecipitation, 1  $\mu$ g of anti-SV40 T antibody was added to 293T cell lysates (100  $\mu$ g), and the mixture was incubated at room temperature for 20 min with end-over-end rotation. As a negative control, lysate was incubated without the anti-SV40 T antibody. The IP was then carried out, in duplicate, using Capturem Protein A. Eluted sample was resolved on a gel and transferred to a PVDF membrane. Then, the blots were probed with a mouse monoclonal antibody against SV40 T, stripped, and then also probed with a mouse monoclonal antibody against p53.

## Conclusions

Combining optimized IP buffers with the incubation- and resin-free workflow made possible with Capturem Protein A technology, the Capturem IP & Co-IP kit streamlines IP experiments down to just five minutes of hands-on time while maintaining excellent results. The protocol's speed saves antibody-protein complexes from possible degradation and/or loss of activity that might result from lengthy resin-based procedures. Additionally, the system has the flexibility to handle a wide range of projects, for both academic and pharmaceutical research.

## Methods

### Immunoprecipitation performed with Capturem IP & Co-IP Kit

NIH3T3 cell lysates were incubated with 1  $\mu$ g of rabbit polyclonal PP2A B subunit antibody (Cell Signaling) for 10 min at room temperature with end-over-end rotation. Capturem Protein A columns were equilibrated with 400  $\mu$ l of equilibration/loading buffer (1.0 M glycine, 2 M NaCl, pH 9.0), and centrifuged at 1,000g for 1 min. The antibody-lysate complex was then diluted to 400  $\mu$ l in lysis buffer and applied to the spin columns, followed by centrifugation at 30g for 4 min. The columns were then washed with 100  $\mu$ l of wash buffer (PBS) and centrifuged at 1,000g for 1 min. Elution was carried out in 100  $\mu$ l of elution buffer (0.1 M glycine, pH 2.5), with 10  $\mu$ l 1 M tris, pH 8.5 in the collection tube, with centrifugation at 1,000g for 1 min. The various fractions were then resolved by gel electrophoresis, transferred onto

PVDF membranes, and probed with rabbit monoclonal anti-PP2A B antibody (Cell Signaling). Note: The buffers used in this experiment are early formulations of the optimized buffers in the complete Capturem IP & Co-IP kit.

### Examining compatibility of Capturem Protein A columns with a variety of buffers

NIH3T3 cell lysates (100  $\mu$ l, containing 130  $\mu$ g of total protein) were incubated with 0.6  $\mu$ g of PP2A B subunit antibody in the IP buffers provided with either Active Motif (A) or Thermo Fisher Scientific (B) IP kits, or the Promega lysis buffer (C), bringing the total volume up to 400  $\mu$ l. The antibody-lysate mixture was allowed to incubate at 4°C for 1 hr and was then loaded on Capturem Protein A columns that had been equilibrated with 100  $\mu$ l of the corresponding IP buffer. The columns were centrifuged at 1,000g for 1 min, washed with 100  $\mu$ l of wash buffer, and centrifuged at 1,000g for 1 min. The bound immunocomplexes were then eluted with 30  $\mu$ l of either low pH buffer (0.1 M glycine, pH 2.5) (A and C) or Thermo Fisher Scientific low pH elution buffer (B) by centrifugation at 1,000g. The elution process was repeated a second time to ensure complete elution of antibody complexes. Immunoprecipitations were also done using the Active Motif and Thermo Fisher Scientific IP kits following their respective manufacturers' protocols, starting with the same amount of lysate and antibody as used for Capturem columns. All elution samples were then resolved on a gel, transferred to PVDF membranes, and probed for the PP2A B subunit using an appropriate antibody (Cell Signaling). Note: The buffers used in this experiment are early formulations of the optimized buffers in the complete Capturem IP & Co-IP kit.

### Co-immunoprecipitation of p53 and SV40 T antigen from 293T cells

Lysates were prepared from 293T cells expressing both p53 and SV40 T. 1  $\mu$ g of anti-SV40 T antibody (rabbit polyclonal, V-300, SCBT) was added to 293T cell lysates (100  $\mu$ g), and the mixture was incubated at room temperature for 20 min with end-over-end rotation. As a negative control, lysate was incubated without the anti-SV40 T antibody. The IP was then carried out, in duplicate, using the complete Capturem IP & Co-IP Kit. Eluted sample was resolved by gel electrophoresis and transferred to a PVDF membrane. The blots were then probed with mouse monoclonal antibodies against SV40 T (SCBT), stripped, and then also probed with mouse monoclonal antibodies against p53 (SCBT).

[View web page >>](#)

[http://www.takarabio.com/US/Support/Applications/Antibody\\_Purification/IP\\_and\\_Co-IP\\_Using\\_Capturem\\_Protein\\_A](http://www.takarabio.com/US/Support/Applications/Antibody_Purification/IP_and_Co-IP_Using_Capturem_Protein_A)

This is a reprint from a page on our web site. All license, copyright, and trademark information pertaining to this content applies as stated in the original web content. This information can be found at [www.clontech.com](http://www.clontech.com).