High Performance Immunoprecipitation (HPIP)

Direct IP Method

PhyTip® Protein Columns Enable a Rapid, Convenient Direct IP Workflow

In biomedical research, it is often necessary to isolate specific proteins from natural sources in order to study them. One of the most commonly used methods to isolate proteins from their biological sources is immunoprecipitation (IP). In IP, proteins from cellular lysate, serum, or other biological fluids are bound to a protein A (ProA) or protein G (ProG) affinity resin using a bridging antibody. The antibody's antigen binding site binds to the protein of interest, while the Fc chain of the antibody binds to ProA or ProG. After washing nonspecifically bound proteins away, the antibody-protein complex is eluted from the resin prior to further analysis.

Key applications that utilize IP include

- Isolating a protein to determine its molecular weight or physicochemical properties.
- Understanding if a protein has been post-translationally modified.
- Testing whether a protein is produced by a specific tissue, or cell type.
- Probing if a specific protein is expressed upon treatment of an organism or cell with a specific condition (i.e. the presence/absence of a drug) using pulse-chase experiments.

High Performance Immunoprecipitation (HPIP) is a new process that provides key benefits to the researcher performing IP experiments including

- Producing replicable protein bands of higher intensity on SDS-PAGE gels, indicating high quality data.
- Very low background obtained by efficient washing of contaminating proteins from the bound antibody-antigen complexes, critical for obtaining accurate conclusions.
- Highly concentrated immunoprecipitated proteins providing a stronger signal compared with competing techniques, reducing the likelihood of repeating experiments.
- Fast processing of samples with the option of simultaneous analysis of up to 12 IPs at a time.

HPIP and method flexibility

Depending on the user's preference, HPIP can be used for either the direct or indirect IP method.

Direct IP Method

In the direct IP method, a specific antibody is bound to a PhyTip ProA or ProG column in an initial capture step. After binding, excess antibody is rinsed from the resin prior to applying the sample containing the antigen protein target. The immobilized antibody captures the target protein antigen and the resin is then washed to remove nonspecifically bound proteins. After washing, the purified antibody-protein complex is eluted from the resin.

Each step of the process is reproducibly controlled by a robotic liquid handler maintaining specific aspiration and dispense rates/volumes for the different liquids passing over the resin bed in the PhyTip column tip.

Direct IP is preferred when the binding kinetics of antigen protein to antibody are rapid.

Direct IP was carried out using the following samples:

- 10 µg of α-GST antibody in 200 µL of capture buffer.
- 5 µg of GST-tagged antigen protein spiked into 125 µg of total E. coli protein in another 200 µL aliquot of capture buffer.

Direct IP requires equilibration, capture, wash, and elution steps. For each step, the appropriate buffers, or protein

mixtures were pipetted into the appropriate rows of a deepwell plate.

Below, each step and its purpose are described.

- Equilibration (well 1) – PureSpeed tips are shipped in a buffer containing glycerol, so the resin was washed with 200 µL of capture buffer in preparation for antibody capture. Two cycles were carried out using the "high" cycling speed on the PureSpeed program. Equilibration time: 3 minutes.
- Capture 1 (well 2) – 10 µg of α-GST antibody in 200 µL of capture buffer was moved back and forth over the resin bed in the PureSpeed PhyTip® column. For the PureSpeed program, five pipeting cycles were carried out using the "medium" cycling speed. This accomplished the immobilization of antibody to the resin. This step took 11 minutes and 15 seconds.
- Capture 2 (well 3) – Following the first capture step, the resin was washed to remove unbound antibody molecules, as these might interact with the antigen protein and prevent its immobilization. Here, 200 µL of capture buffer was applied to the resin. Five pipetting cycles were carried out using medium cycling speed. The duration of this step was 11 minutes and 15 seconds.
- Capture 3 (well 4) – 200 µL of solution containing 5 µg of GST-tagged antigen protein and 125 µg of E. coli total protein in capture buffer was moved back and forth over the resin bed. The GST-tagged antigen protein binds to the captured antibody already on the resin. Here, five pipetting cycles were used with medium cycling speed. After cycling, the resin had both antibody and antigen proteins bound. This step required 11 minutes and 15 seconds.
- Wash 1 (well 5) – To remove potential contaminant proteins, the resin was washed with capture buffer. Here, 200 µL of buffer was used with a single, medium speed cycle. This step was completed in 2 minutes and 15 seconds.
- Wash 2 (well 6) – A second wash step was included to further remove contaminants. Again, 200 µL of capture buffer was used in a single, medium speed cycle. This step was also completed in 2 minutes and 15 seconds.
- Elution (well 7) – The last step in the protocol was the elution of the antibody and antigen proteins. 40 µL of acidic enrichment buffer was applied to the resin. Five pipetting cycles, carried out at "medium" speed, were used. This final step required 6 minutes and 50 seconds. After completion of the protocol, the antibody- antigen sample was collected from the deep well plate and neutralized by adding a ¼ volume of PureSpeed neutralization buffer.

Total processing time for the HPIP direct method: ‹ 50 minutes

SDS-PAGE Analysis

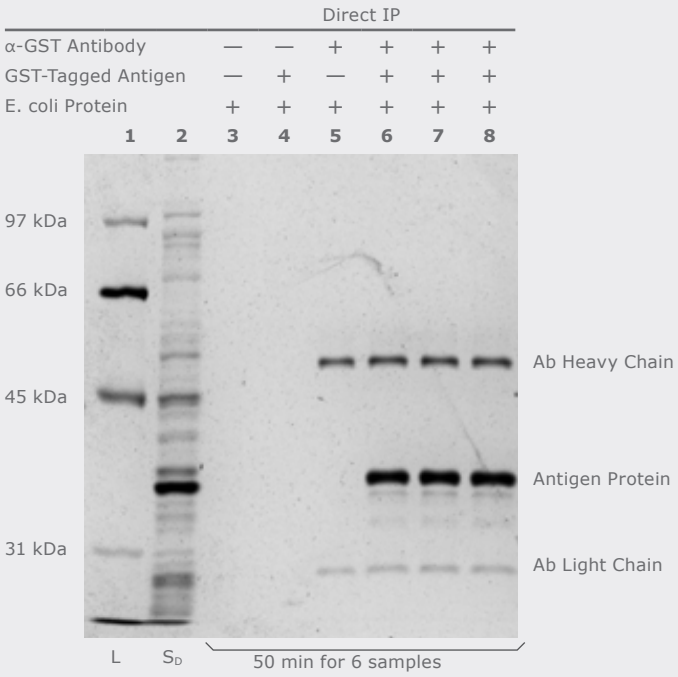
Elute samples and pre-immunoprecipitated lysate were mixed with 5 X Sample Loading Buffer (National Diagnostics) prior to heating at 80 °C for 10 minutes. The samples were briefly centrifuged and then loaded onto a 10 % Polyacrylamide Tris-Glycine Gel (Novex). After electrophoresis, the gels were silver stained for band visualization (Figure 1).

Figure 1. Direct IP with ProG PhyTip® columns

Direct IP was carried out as described in the text. Pluses and minuses indicate the inclusion or exclusion of antibody, antigen protein or both within a given sample. L and S_D denote the molecular weight ladder and protein sample containing the antigen protein, respectively. The time needed for the protocol is noted beneath the gel. Load volumes were 5 µL for all samples.

Gel Legend

1. Ladder
2. PureSpeed Protein Sample Containing Antigen
3. PureSpeed Direct IP: — Antibody; — Antigen Protein
4. PureSpeed Direct IP: — Antibody; + Antigen Protein
5. PureSpeed Direct IP: + Antibody; — Antigen Protein
6. PureSpeed Direct IP: + Antibody; + Antigen Protein
7. PureSpeed Direct IP: + Antibody; + Antigen Protein
8. PureSpeed Direct IP: + Antibody; + Antigen Protein



Summary

The PureSpeed HPIP system brings efficiency, robustness and ease to IP protocols, demonstrating a direct IP protocol in less than 50 minutes. The data is highly reproducible: three replicates show similar data for the direct method.
The semi-automated format of PureSpeed and E4 XLS electronic pipette reduces the amount of time the user needs to pipette.

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