# High Performance Immunoprecipitation (HPIP)

## Indirect IP Method

### PhyTip® Protein columns enable a rapid, convenient Indirect 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.

### Indirect IP Method

In the indirect IP method, antibody and antigen protein-containing sample are premixed prior to applying to ProA or ProG column. The resin binds the antibody-antigen protein complex in one step and after washing the resin, the antibody-antigen proteins are eluted.
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 tip column.

### Indirect IP Method using High Performance Immunoprecipitation

The indirect method is preferred when the binding kinetics of antigen protein and antibody are slow, when there is concern that shear forces could disrupt a protein complex, or when a researcher plans to eventually carry out co-immunoprecipitation.

Indirect IP was carried out using the following sample:

- 10 µg of α-GST antibody, 5 µg of GST-tagged antigen protein spiked into 125 µg of total E. coli protein in a
- 200 µL aliquot of capture buffer which was incubated overnight at 4° C to produce the antibody/protein complex.

Indirect IP requires separate equilibration, capture, wash, and elution steps. For each step, the appropriate buffers or protein mixtures were pipetted into the appropriate wells in the deepwell plate.

Below, each step and its purpose are described:

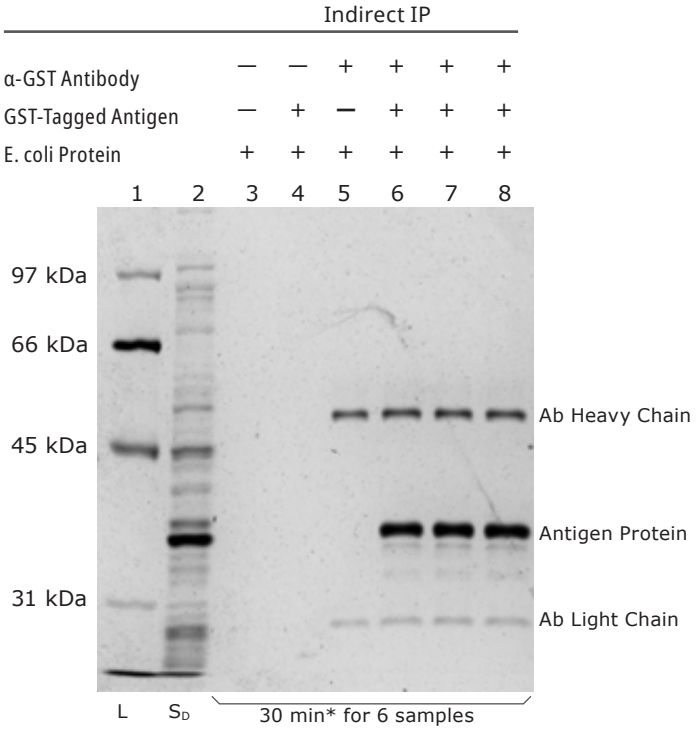
- Equilibration (well 1) – PhyTip columns are shipped containing glycerol, so the resin was washed (equilibrated) with 200 µL of capture buffer in preparation for antibody-antigen protein capture. Two cycles were carried out. Equilibration time: 3 minutes.
- Capture (well 2) – After the equilibration step, the 200 µL of antibody/protein complex was moved back and forth over the resin bed of the PhyTip protein tip. Five pipetting cycles were carried out using the "medium" cycling speed. This accomplished the immobilization of antibody and antigen protein to the resin. Capture time: 11 minutes and 15 seconds.
- Wash 1 (well 3) – Resin was washed to remove contaminant proteins. 100 µL of capture buffer was applied to the resin. One pipetting cycle was carried out using medium cycling speed. Wash 1 time: 1 minute and 30 seconds.
- Wash 2 (well 4) – The resin was separately washed a second time using 100 µL of capture buffer in a single, medium speed pipetting cycle. Wash 2 time: 1 minute and 30 seconds.
- Wash 3 (well 5) – The resin was washed a third time with capture buffer. Again, 100 µL of capture buffer was used with a single, medium speed cycle. Wash 3 time: 1 minute and 30 seconds.
- Elution (well 6) – The last step in the protocol was the elution of antibody and antigen proteins from the resin. 40 µL of acidic enrichment buffer was applied to the resin. Five pipetting cycles, carried out. This final step required 7 minutes. After completion of the protocol, the antibody-antigen sample was collected from the deepwell plate and neutralized by adding a neutralization buffer.

Total processing time for the HPIP Indirect method: 26 minutes

## SDS-PAGE Analysis

Eluate 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.

**Indirect IP with ProG PhyTip® columns**




Indirect 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 SI denote the molecular weight ladder ladder and protein sample containing the antibody and antigen protein, respectively. The time needed for the protocol is noted below the gel. Load volumes were 5 µL for all samples.

*The protocol time does not include overnight incubation of the antibody and antigen protein.

## Experimental Data

### Gel Legend

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