Application Note

High Performance Immunoprecipitation

The PureSpeed System: Indirect IP Method

PureSpeed™ Protein Tips and the E4™ XLS™ pipette 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 F_c 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™

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.

METTLER TOLEDO

HPIP uses a Rainin E4 XLS electronic pipette and PureSpeed Protein tips containing either ProA or ProG resin to perform reproducible IP experiments. Other components of the system include a 96-deepwell plate, adapters, deepwell plate base and ColorTrak™ guide, shown in Figure 1.

Figure 1: E4 XLS with Purespeed Tips and accessories

The E4 XLS pipette can be programmed to carry out semi-automated liquid processing for IP applications. The system provides high flexibility allowing the user to enter the solution volume, pipetting speed, aliquot number, and cycle number for the resin equilibration, sample capture, wash, and elution steps.

HPIP and method flexibility

Depending on the user's preference, HPIP can be used for either the direct or indirect IP method (for more information on the direct approach, please see the "High Performance Immunoprecipitation. The PureSpeed System: Direct IP Method" application note).

Indirect IP Method

In the indirect IP method, antibody and antigen protein-containing sample are premixed prior to applying to ProA or ProG resin. 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 the E4 XLS pipette – maintaining specific aspiration and dispense rates/volumes for the different liquids passing over the resin bed in the PureSpeed tip.

Here, indirect IP with PureSpeed is demonstrated using the protocol below.

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.

With the HPIP system, the affinity resin used to capture the resin is retained at the distal end of a tip (see PureSpeed ProA and ProG data sheet for details) and liquid flow over the resin is controlled by an E4 XLS electronic pipette which is available not only as a single channel system but also a multi-channel system (up to 12 channels) that enables parallel processing of samples.

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 different wells in the deepwell plate prior to starting the PureSpeed program on the E4 XLS electronic pipette.

Below, each step and its purpose are described:

Equilibration (well 1) – PureSpeed tips 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 using the "high" cycling speed on the PureSpeed program. 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 PureSpeed protein tip. For the PureSpeed program, 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 at "medium" speed, were used. 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 ¼ volume of PureSpeed 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.

Experimental Data

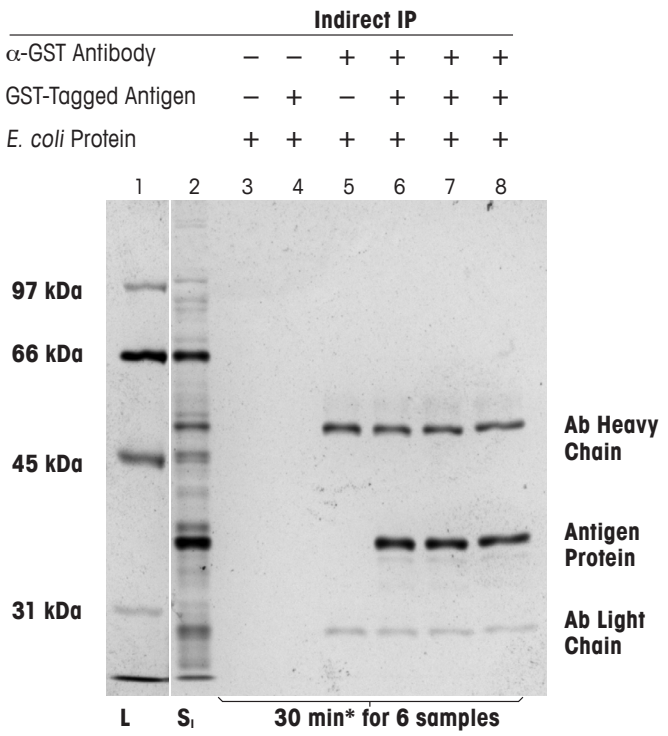
Gel Legend

1 Ladder
2 PureSpeed Protein Sample Containing Antibody and 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

Load volumes were 5 µL for all samples.

Summary

The PureSpeed HPIP system brings efficiency, robustness and ease to IP protocols, demonstrating an indirect IP protocol in less than 30 minutes. The data is highly repro ducible: three replicates show similar data for the indirect method. The semi-automated format of PureSpeed Protein tips and E4 XLS electronic pipette reduces the amount of time the user needs to pipette.



Indirect IP with PureSpeed ProG Tips

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 **S_I** 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.

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

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