SPOTLIGHT feature

Proteomics, Genomics & Microarrays

Discovering Hidden Protein Interactions

Capturing Weak Protein-Protein Interactions with Exclusion-based Sample Preparation

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Identifying weak or transient protein-protein interactions has the potential to reveal new targets for drug discovery. These interactions affect cellular processes including growth, signaling, morphology, and death, but common methods of protein purification leave much to be desired when attempting to study weak or transient interactions.

Exclusion-based Sample Preparation (ESP™) is a new method of protein purification that can accelerate experimental workflows while preserving weak protein interactions for subsequent analysis.

Interest in identifying specific protein-protein interactions as therapeutic or diagnostic targets has grown with the understanding of their roles in cellular processes [1]. Protein-protein interactions influence most cellular processes, including gene expression, cell growth, signal transduction, morphology, and apoptosis. Disrupting these interactions can offer a new and effective strategy for combating cancer and other diseases that rely on the transmission of signals.

Unfortunately, identifying appropriate protein-protein interaction targets has proven difficult due to their biological complexity [2]. This challenge is especially pronounced in the study of low-affinity or transient interactions [3], which are difficult to examine experimentally due to how easily they dissociate during wash steps of conventional protein purification methods [4].

The ability to isolate and identify endogenous protein-protein interactions has the potential to increase drug specificity, improving therapeutic outcomes and reducing problems with toxicity. However, that potential can only be realised if researchers can capture weak or transient interactions for analysis.

A new approach to affinity purification, Exclusion-based Sample Preparation (ESP), allows researchers to capture weak or transient protein-protein interactions for downstream analysis. The ability to capture these interactions could lead to new understanding of a variety of cell processes, including, for example, the rapid interplay between transcription factors and RNA polymerase during transcription.

Conventional Methods of Protein Purification

The development of affinity purification advanced the study of proteins and protein-protein interactions. With affinity purification procedures, such as affinity chromatography and co-immunoprecipitation, researchers can use biological interactions between target proteins and specific molecules to separate the desired analyte from a complex sample. Improved ability to target specific analytes led to purer and more easily analysed samples [5].

The fundamental principle behind affinity purification is simple: certain molecules or compounds have highly specific interactions with a protein. By introducing those molecules into a sample, usually affixed to a solid matrix such as a resin or bead, proteins of interest can be captured from the rest of a solution.

The process of affinity purification was streamlined with the advent of paramagnetic beads. Using a solid support that responds to a magnetic field makes the process more efficient, as researchers can simply use a magnet to hold the beads in place and draw off the solution with a pipette. When compared to non-magnetic agarose or sepharose beads, which rely on slow diffusion during the wash and incubation steps, paramagnetic beads greatly accelerate affinity purification and contribute to a gentler overall experimental procedure [6].

Although advances in affinity purification, including the use of paramagnetic beads, have accelerated the process to a degree, researchers are still plagued by two fundamental challenges: the inability to detect weak and fleeting protein interactions and slow workflows due to the speed of purification.

Multiple harsh wash steps involved in most affinity purification techniques limit the ability to detect weak or transient protein interactions. These wash steps include buffers that promote protein dissociation, leading to the loss of weak and transient protein interactions that play important roles in cellular processes.

In addition, purification done in tube-based workflows still requires a separate tube for every protein target being analysed. Because experiments can require several purification steps, repeating every step for every sample tube significantly lengthens timelines and reduces efficiency.

More Sensitive Purification through Exclusion-based Sample Preparation

The limitations of affinity purification for the study of weak protein-protein interactions necessitates the development and use of different, gentler affinity purification techniques. The conventional process isolates the analyte by removing the wash solution. By inverting this process and extracting the target analyte from the wash instead, Exclusion-based Sample Preparation (ESP) has fewer and gentler wash steps and improves researcher's ability to detect weak or transient protein-protein interactions.

Removing analyte from solution, rather than flushing solution away from the analyte, is a subtle but critical difference between ESP and traditional affinity-based approaches. Because the original sample is left intact, the analyte is left with less debris that could impact the binding of low-affinity proteins.

Less sample debris similarly leads to a reduction in the number of required wash steps. Not only does this avoid exposing the analyte to unnecessary wash buffers, which can cause proteins to dissociate, but weak or transient protein-protein interactions are more likely to persist in the absence of harsh, pipette-based washing. ESP allows gentle, complete wash steps to be executed in seconds, reducing purification bottlenecks for experimental workflows.

These time savings are even more pronounced in the first commercialisation of ESP as Gilson's Extractman®. Unlike tube-based procedures, which require samples to be run in individual tubes, this ESP technology runs samples in parallel for greater throughput.

Detecting Protein-Protein Interactions with ESP

A study comparing ESP with the traditional co-immunoprecipitation protocol for the separation of GST-tagged proteins demonstrated that ESP allowed the capture of an additional protein as evaluated by subsequent Western blot analysis [7].

Four proteins were examined using both methods: Maltose Binding Protein (MBP), Glutathione-S-Transferase (GST), an MBP fusion with Unidentified Protein A (MBP-UPA) and GST fusion with Unidentified Protein B (GST-UPB). Researchers then ran sample supernatants on SDS-PAGE gels using an anti-GST antibody. Results demonstrated that the ESP workflow led to the detection of an interaction between UPA, the bait protein, and UPB, the prey protein. The standard Co-IP procedure, on the other hand, was not able to reveal this interaction *(Figure 1)*.

The ESP procedure used in Extractman is fast and easy. Researchers simply need to slide the handle of the device to move the target analyte through binding, wash, and elution steps. No pipetting necessary.

ESP procedure begins similarly to other affinity purification methods: a sample containing the target analyte is combined with paramagnetic beads and an appropriate reagent. After this point, however, procedures begin to diverge. Rather than using magnets to separate the target analyte in tubes, ESP uses a magnet that passes over a well containing the sample.

As the magnet moves over the well, it attracts the paramagnetic beads with bound analyte onto a disposable bead capturing strip. Because the analyte is extracted from the sample, the remainder of the sample is left intact for any further desired analysis. The use of a disposable strip prevents cross contamination between experiments.

With analyte bound and attracted, the sliding handle is used to move the beads with bound analyte to the next set of wells for wash steps. A magnet below the wells engages to repel the first magnet and allows the beads to lower into the well for washing. After repeating this process through the appropriate number of washes, the upper magnet collects the now-washed analyte and deposits it in the final well for elution *(Figure 2)*.

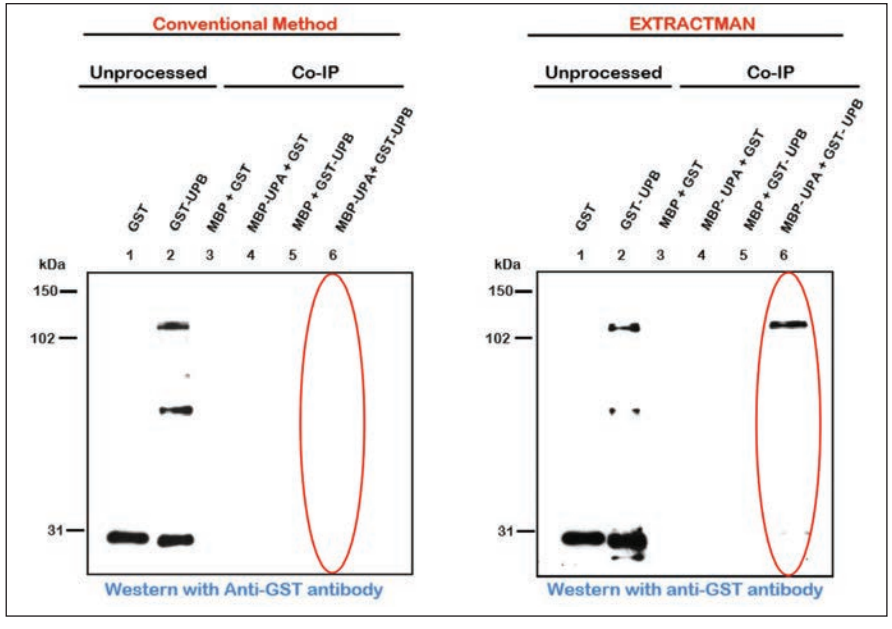

Figure 1. Results of western blot analysis examining Maltose Binding Protein (MBP), Glutathione-S-Transferase (GST), an MBP fusion with Unidentified Protein A (MBP-UPA) and GST fusion with Unidentified Protein B (GST-UPB). The procedure revealed a potential interaction between UPA and UPB.

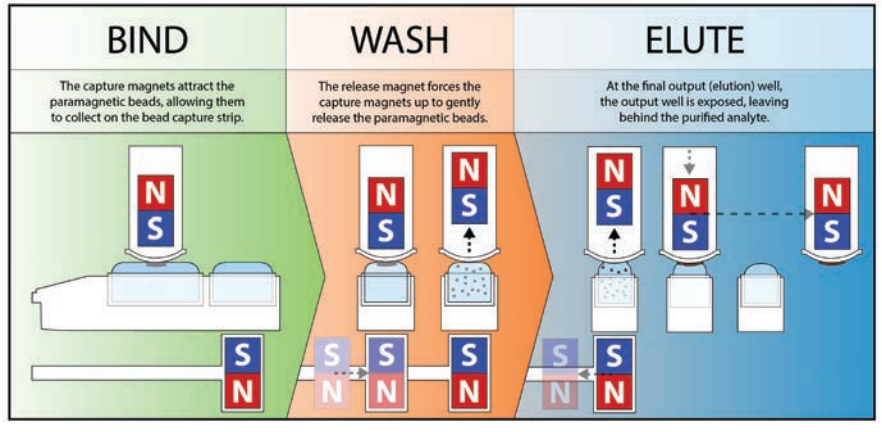

Figure 2. The interplay of the two magnets in Gilson's EXTRACTMAN along with the surface tension of the buffers used allows for the movement of paramagnetic beads through through the bind, wash and elute steps without disturbing the droplets. N and S represent the magnetic poles.

By gently transferring the bound substrate from well to well rather than purify a sample in tubes, ESP minimises harsh wash steps and reduces time spent in wash solutions that cause protein dissociation. These changes both accelerate workflows and allow the detection of weak or transient protein-protein interactions, making ESP the ideal affinity purification method.

References

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