SPOTLIGHT feature

Laboratory Consumables

FPLC in a Pipette Tip

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The humble pipette tip has come of age. Now not just for transferring your sample, new functional tips actually perform your assay. Protein purification tips enable affinity purification that gives you higher concentration product with superior purity, faster and easier than ever before.

Many thousands of disposable pipette tips are used every day in laboratories worldwide. These simple plastic consumables may seem insignificant, but their quality plays a critical role in ensuring accurate and precise liquid dispensing. In addition to using a precision pipette that is regularly calibrated and being trained in correct pipetting technique, using pipette tips made in quality moulds from flexible virgin polypropylene is essential. The purity of the tip should also be considered as tips that are not BioClean could lead to contamination of samples or assay interference.

However, today the humble pipette tip is not just for transferring samples. Advances in technology have created a new generation of functional tips that actually contain and perform your assay. This new technique has significant advantages over traditional methods, for example for protein purification, providing a superior end product in less time, yet remaining a low cost solution.

Protein Purification

A variety of methods are traditionally employed for purifying proteins and other macromolecules of interest from crude extracts or other complex mixtures. Most techniques, however, involve some form of chromatography whereby molecules in solution (mobile phase) are separated based on differences in chemical or physical interaction with a stationary material (solid phase). Affinity chromatography (also called affinity purification) makes use of specific binding interactions between molecules. A particular ligand is chemically immobilised or 'coupled' to a solid support so that when a complex mixture is passed over the column, those molecules having specific binding affinity to the ligand become bound. After other sample components are washed away, the bound molecule is stripped from the support, resulting in its purification from the original sample. A range of tools are currently available for affinity protein purification including: spin columns, gravity columns, FPLC and magnetic beads. Each specific affinity system requires its own set of conditions and presents its own peculiar challenges for a given research purpose.

Protein Tips are novel protein capture devices using various affinity resins maintained at the base outlet of a pipette tip. They form part of a unique purification and enrichment system that includes an electronic pipette to automate and control the process of Capturing, Purifying and Enrichment of proteins. Protein Tips have been optimised to produce the highest possible purity, yield, concentration and activity of sample proteins. In addition, they are able to perform these functions with low volume samples, thus saving valuable resources. Sample work flow is seamlessly integrated with protein expression and assays, with complete sample and solution tracking. The tips can operate rapidly in parallel to perform purification in minimal time, frequently in as little as 15 minutes. They are easy to set up and are disposable after use, making buffer preparation and clean up very easy.

More Efficient Protein Capture, Higher Purity, Greater Concentration

One of the main advantages of using a pipette tip containing affinity resin for protein purification is the repeated bidirectional flow of sample volume through the tip bed, afforded by the electronic pipette controlling the system.

Through recurring aspiration and dispense the back and forth flow delivers greater saturation, stronger binding kinetics and higher protein concentration than either gravity-fed affinity columns or spin columns. Target proteins that are present in the sample are transported actively to the affinity resin bed with sufficient cycles to increase contact time to fully capture the protein.

This active transport process increases the capture kinetics (over diffusion processes) and allows the capture equilibrium reaction to reach completion.

Competitive technologies have less efficient capture processes. Spin columns need a larger resin bed to give a better chance of capture. The flow through a spin column is extremely rapid giving limited time for protein capture in only a single fluid pass. Proteins need sufficient time to interact with the resin to be captured. Increasing the bed size of a spin column bed increases the opportunity for capture but remains an inefficient process.

Gravity columns and FPLC have less control over the fluid flow than the pipette and Protein Tip system. With columns it is impossible to use very small capture volumes. The same is true for the elution step that requires a large elution volume to be effective. These large buffer volumes dilute the recovered protein. Protein tips use very small elution volumes, thus also making this step an enrichment process.

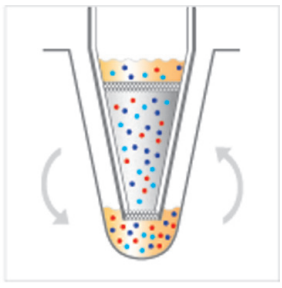
Magnetic beads have low capacity as only the surface is derivatised. They cannot use back and forth flow, so the capture process is slower. The washing/purification process is less effective and so recovered protein is not as pure. For the same amount of magnetic bead resin, smaller elution volumes can be used with Protein Tips making the recovered protein much higher in concentration.

The low volume of resin in a Protein Tip allows a greater wash volume. Combined with repetitive multiple wash steps this efficiently removes unbound, nonspecific proteins and contaminants. A unique second wash step optimises the protein purification process to produce the highest enrichment possible, during the final elution step.

In the final step, a small volume of low pH elution buffer releases the protein from the resin. With this efficient and low volume system, Protein Tips produce the highest concentration of purified material of any purification technique.

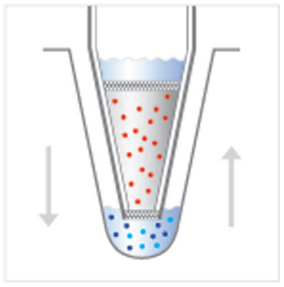
Protein Purification Steps using Protein Tips

Used with the customisable purification protocol on the electronic pipette, processing for up to12 protein samples in parallel, is automated in as little as 15 minutes. Protein Tips eliminate the need for additional concentration steps.



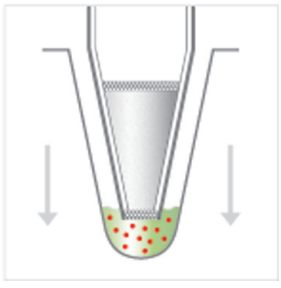
Capture the target protein

The unique, patented design of the packed resin bed retains the affinity resin in the Protein Tip. As sample is aspirated into the tip, target protein binds to the resin, while non-binding proteins and contaminants pass through. The sample is repeatedly drawn through the resin, reaching equilibrium binding.



Purify the protein sample

Similar to the capture step, repetitive multiple wash steps remove unbound, nonspecific proteins and contaminants from the tip, leaving the concentrated target protein bound to the affinity resin bed.



Elute the enriched protein

Repeatedly drawing the elution buffer through the tip moves the concentrated target protein from the resin back into a fresh sample well.

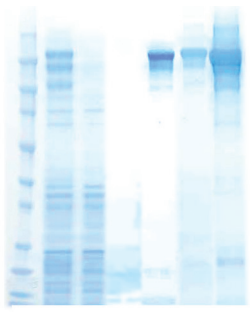
For this phase, a minimal volume of elution buffer is required to release the pure target protein, producing the highest concentration possible.

The Tip Concentrating Effect

The Tip Concentrating Effect allows the recovery of high concentrations of proteins in a single purification procedure [1]. Protein Tips are designed to produce very high concentrations of recovered proteins compared to traditional techniques such as spin columns, gravity columns, magnetic beads and FPLC. They capture protein on very small resin bed volume and recovery is a highly efficient and effective process. All conditions being equal, with any two different resin bed sizes of Protein Tips, the smaller resin bed size will produce the highest concentration of recovered protein.

It may seem counter intuitive to increase the concentration of a protein by using a smaller tip, since with all other technologies the recovered protein becomes more dilute as the resin bed size is decreased. Protein tips are unique: they are designed to use the same elution-volume-to-bed ratio as the bed volume is decreased, usually a 3x ratio. Even with resin bed sizes as low as 5µL (a 100-fold decrease over a 'normal' 0.5mL resin bed) a 3x (or even a 2x) elution volume can be used. Thus, an 80µL bed uses a 240µL elution volume; a 20 µL bed uses a 60 µL elution volume; a 5µL bed uses a 15µL elution volume, and so on. For example, to increase the concentration of a recovered sample, all capture and wash conditions are kept the same but the resin bed volume of the tip is decreased from 80µL to 20µL. Essentially, the same amount of protein is captured with 20µL bed vs. the original 80µL bed, but now is eluted with 60µL of buffer rather than the original 240µL. Thus, the concentration of the recovered protein can be increased up to 4 fold over the original, larger bed size purification process.

NuPAGE gel showing E.coli lysate spiked with 0.5 mg/mL hIgG and purified using Protein tips and spin column.



1 2 3 4 5 6 7

Lane 1: Protein ladder

Lane 2: E.coli lysate spiked with hIgG

Lane 3: Flow through

Lane 4: 100 µL lysate purified on spin column with 200 µL ProA

Lane 5: 100 µL lysate purified on Protein Tips with 5 µL ProA

Lane 6: 500 µL lysate purified on spin column with 200 µL ProA

Lane 7: 500 µL lysate purified on Protein Tips with 20 µL ProA

Optimising and Enhancing the Capture and Elution step

Resin beds are loaded with a very high percentage of available binding sites and substantial amounts of protein can be captured with small bed volumes. Since only very small elution volumes are needed to elute the proteins from the resin bed, the recovered protein concentration is very high. As the elution volume is decreased, the concentration of protein is increased. The concentrations of recovered proteins are 5-20 times higher than what can be produced from competitive technologies.

Affinity Resins

Affinity resins are designed to be selective to a particular protein tag, property or characteristic. Recombinant proteins are normally purified using a tag on the protein and an affinity resin that is selective to the particular tag. His-tagged proteins are purified on IMAC affinity resins. Antibodies are purified with Protein A or Protein G affinity resins. The combination of affinity and specificity has been exploited to generate straightforward affinity purification methods. Protein tips are available with ProA, ProG and IMAC resins.

What Size Proteins?

Most researchers study proteins in the 5 to 200 kDa size range. Interestingly as the protein size increases, the larger, more bulky proteins may not stick as tightly to the affinity resin and the conditions for purification are usually less stringent to prevent loss of protein. Nevertheless, these proteins are purified quite easily. Protein complexes, however, are much larger (for example, 1 MDa to perhaps the 8 MDa range).

Proteins in a complex held in a strong core of proteins (proteins tightly associated with each other) are usually captured by packed bed columns and will also be captured by the Protein Tips.

Transient (non-core) proteins are often difficult to isolate in a packed bed system; they are often too large and fragile to survive the process of flowing through the packed bed. The gentle action of the Protein Tips system may enable purification of transient proteins in protein complexes. Each system should be examined on a case-by-case basis.

Overcoming the Challenges of Affinity Purification

Affinity purification methods often struggle to maintain or increase protein concentration while, at the same time, assuring the protein is pure and active. This is especially true as the amount or volume of protein being purified is decreased. It can be very difficult to manipulate microlitre volumes of sample. Finally, it is difficult to routinely purify large numbers of samples in parallel in a laboratory environment.

Protein Tip technology offers a new solution to easily overcome all these challenges.

Reference

1. D. T. Gjerde, Concentrating Effect of Pipette Tip Columns, submitted (2012).

Sporicidal Wipes you Can Actually See Work

Tristel Sporicidal Surface Wipes are the latest addition to the 'Tristel for Surfaces' product range and provide a practical and highly effective way to decontaminate all hard surfaces, including those of non-invasive medical devices and food preparation areas. Quick and easy to use, the Tristel Sporicidal surface wipe uses chlorine dioxide to provide high-level disinfection against all microorganisms (spores, mycobacteria, viruses, fungi, bacteria) in under a minute. Once activated, the wipe changes colour so the user knows it's ready for use. For improved safety, microorganisms are killed on the wipe so they are not simply transferred to another surface during the decontamination process.

The Tristel Sporicidal surface wipe system generates chlorine dioxide when the activator foam is applied to the wipe. An almost instant reaction generates a controlled level of chlorine dioxide in aqueous solution, which is contained within the wipe. The wipe changes from pink to white when the chemical reaction has taken place making it clear that the wipe is ready for use. Incorporated in the Tristel technology is a buffering system that stabilises the pH at close to that of the skin mantle and an inhibitor system that protects sensitive materials. Tristel Sporicidal surface wipes system can be used for the high-level disinfection of commodes, wheelchairs, mattresses, beds, near-patient areas and food preparation areas.

Chlorine dioxide is a powerful oxidising agent and a well-documented, highly effective and safe biocide. Tristel's patented chlorine dioxide chemistry can kill all organisms on a surface from which excess soil and organic matter have been removed, with a contact time of only 30 seconds. Biocidically, the Tristel Sporicidal surface wipe is far superior to a wipe that uses alcohol, a quaternary ammonium compound, a biguanide, chlorhexidine gluconate or any other chemistry. Tristel's chlorine dioxide is completely and rapidly biodegradable.