

## Improve the Efficiency of Your Protein Purification

### Introduction

Purifying proteins is essential to investigating their properties. There are many purification methods available using protein tags and chromatography. **Protein tags** are peptide sequences genetically grafted onto a recombinant protein. Tags are attached to proteins of interest to allow proper separation by chromatography to enhance the key protein for research or replication.

### Theory

In this study, polyhistidine-tags also known as “hexa histone-tags” or “HIS tags” are used along with metal ion affinity gravity column chromatography to facilitate the purification process. HIS tagging is the option of choice for purifying recombinant proteins in denaturing conditions because the separation or purifying technique does not negatively impact the primary structure of the protein in question. This makes it easier to purify or separate the desired protein.

The HIS tagging protein technology is widely used because the tags are small, insoluble and rarely interfere with the function, activity, or structure of the wanted protein. The desired protein is genetically engineered to have six consecutive histone amino acids inserted at the beginning of the protein. The six amino acids are designed to firmly bind to nickel or cobalt ions used in the purification media using immobilized metal ion affinity chromatography.

Immobilized metal ion affinity chromatography (IMAC) is the most common method for purifying histidine-tagged proteins. This method requires an IMAC media containing bonding metal ions, such as cobalt, that will selectively retain histidine-tagged proteins. The media is placed into a chromatography column. A low imidazole concentration buffer solution is used to carry the HIS tagged proteins. This buffer is slowly infused through the chromatography column to allow the HIS tagged proteins to bind to bonding cobalt ions contained in the IMAC media. Then the tagged proteins are eluted from the media using a high imidazole concentration buffer.

This method allows for reliable purification of histidine-tagged proteins from the media when a controlled volume and flow of denaturing buffers such as imidazole are infused into the IMAC column. The more controlled the purification process, the higher yield of purified protein will be recovered from one pass through the column. A controlled and repeatable IMAC purification process gives the researcher a high yield of pure and active target protein in the shortest amount of time.

The following application gives example of this efficient purified protein extraction process.

### Application

Many laboratories seek an economical method to perform efficient protein purification. One convenient method is to simultaneously deliver two independent imidazole buffers through a cobalt gravity column in a precise and controlled manor for purification of their HIS tagged proteins. (Figure 1).

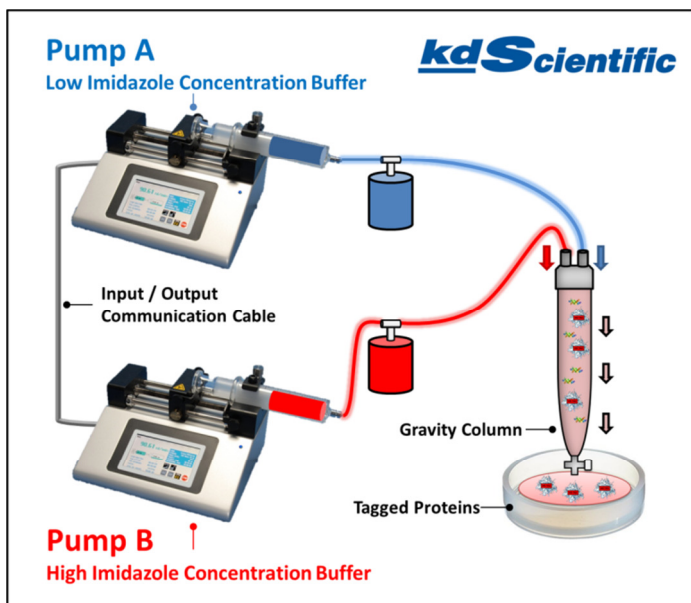


Figure 1: Diagram of imidazole infusion into a gravity column

The ideal syringe pump solution for this application is the **KD Scientific Legato 110 Dual Rate System (Legato 110 DRS)**. The **Legato 110 DRS** is an innovative syringe pump system that allows the user to easily configure two different syringe pumps with independent flow rates using the built in multistep programming and input / output signal communication. All control is set through the innovative Legato touch screen interface. No external programming or computer is required.

For this application, the first pump (Pump A), controls a 10 ml gas tight syringe that will first be used to infuse 10 ml of a low imidazole concentration buffer containing the HIS tagged proteins through the gravity column at a flow rate of 1 ml/min for 10 ml (10 minutes). The precise flow rate is required to have proper HIS tagged protein bind to the cobalt IMAC particles in the column.

Pump B controls a 10 ml gas tight syringe that will infuse a high imidazole concentration buffer required to elute the tagged proteins off of the cobalt column.

The next step requires Pump A to refill by having the pump operate in withdraw mode. Pump A sends a signal to Pump B, allowing Pump B to fill its syringe with a high imidazole concentration buffer. The change in pressure from infuse to withdraw will allow a check valve to open to fill both pumps with the proper imidazole concentration buffer. The withdraw needs to be quick but controlled as to not allow the buffer to degas. In this application, degassing the buffer would have a negative impact on the purifying chemical reaction in the cobalt gravity column.

Once both syringes are filled, Pump A and Pump B will start their programmed flow profiles simultaneously, creating a flow gradient in the cobalt gravity column. Pump A is infusing the low concentration buffer starting at 1 ml/min and ending at 0 ml/min over a 20 minute time span. (Figure 2) Pump B infuses the high concentration imidazole buffer with a flow rates starting at 0 ml/min and ending at 1 ml /min over 20 minutes (Figure 3).

This method allows the total flow through the column to remain at 1 ml/min during any point during the gradient. This rate allows proper flow to elute the tagged proteins without over pressurizing the gravity column. (Figure 4).

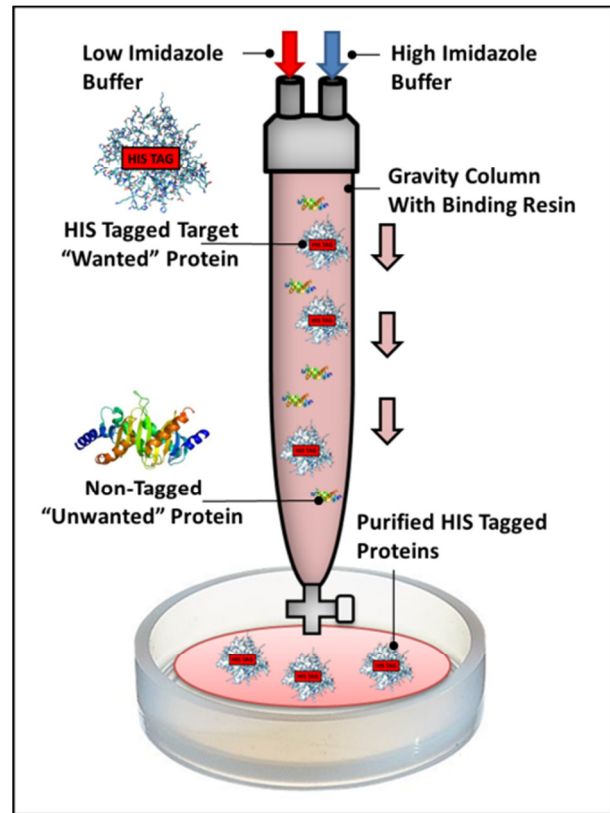


Figure 4: Column gradient flow

#### Benefits of the Legato 110 DRS

- ✓ Easy to use with intuitive touch screen display.
- ✓ Prepare and elute samples with one simple process.
- ✓ Precise and controlled dual independent flow rates.
- ✓ Low cost solution when compared to expensive chromatography systems.
- ✓ Repeatable results.
- ✓ **KD Scientific** provides world class technical support for all of our products.

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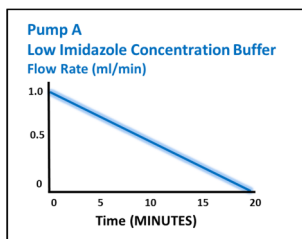


Figure 2: Pump A Flow Profile

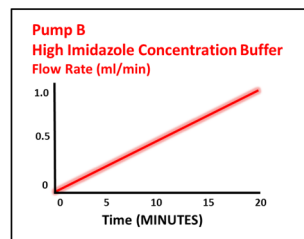


Figure 3: Pump B Flow Profile