APPLICATION NOTE

His Tag Protein Production and Purification

INTRODUCTION

The study of protein regulation, structure, and function relies heavily on the expression and purification of recombinant proteins. Many recombinant proteins are expressed as fusion proteins, meaning that they contain an affinity / epitope tag. A tag is a short sequence of DNA that codes for a specific amino acid, which is frequently inserted into a target gene at the point of coding for expression at either the N or C terminal of the protein required.

One of the most commonly used tags is the polyhistidine tag, also known as His Tag, which is a string of between six and nine histidine residues (see Figure 1 below). This method of tagging is especially useful as it allows for easy purification and detection of the recombinant protein.

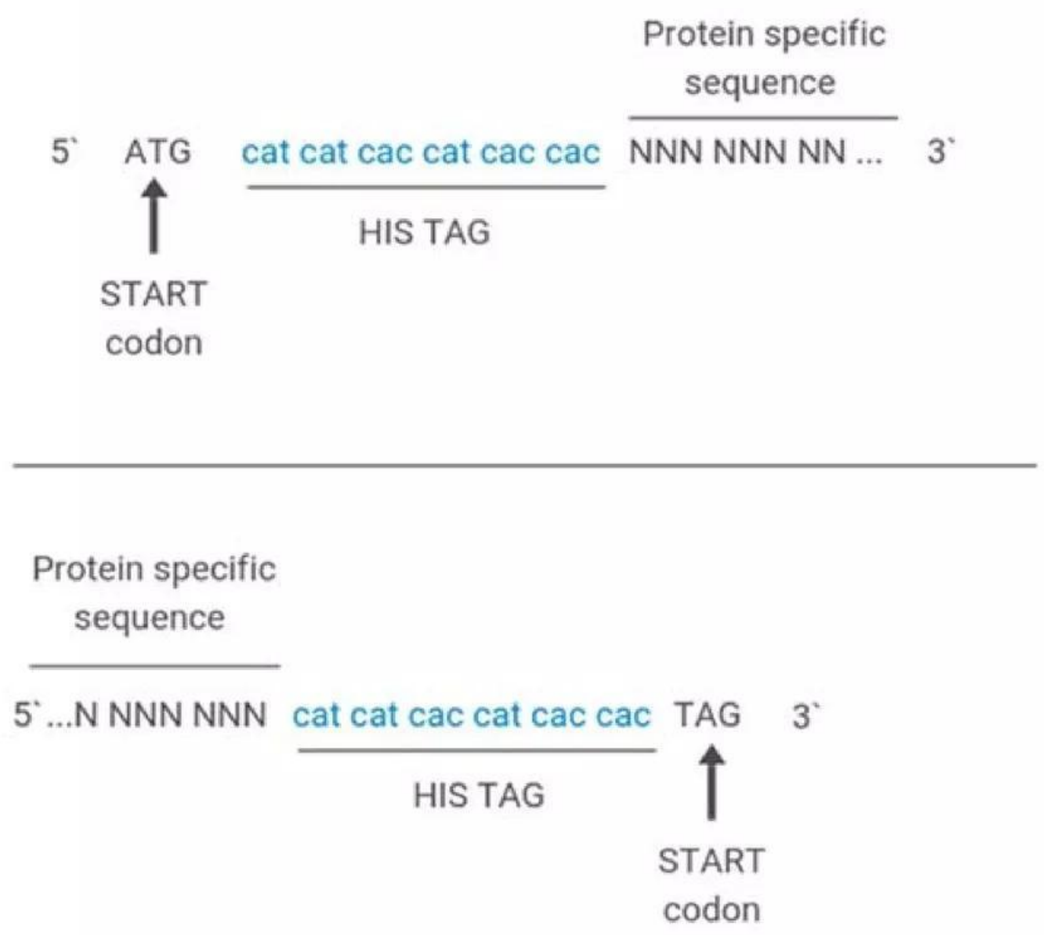

Figure 1. His Tag Fused N or C Terminal of the Recombinant Protein.

PURIFICATION OF HIS TAG PROTEINS

Protein purification requires methods that are highly specific and robust, regardless of scale. His tags allow researchers to selectively extract a protein of interest from the thousands of other proteins found in either a cell or cell lysate. Purification of His Tag proteins is by single step immobilized metal affinity chromatography (IMAC); a specialized form of affinity chromatography where proteins (or peptides) are separated according to their affinity for metal ions immobilized to a solid chelating resin. Although many bivalent metal ions (e.g. Zn^{2+}, $Cu^{2+,}$ Cd^{2+}, Hg^{2+}, Co^{2+}, Ni^{2+}, and Fe^{2+}) can be used in IMAC, nickel (Ni^{2+}) or cobalt (Co^{2+}) are often used in chelating resins to coordinate and selectively bind to the His Tag. Nickel is generally used for higher protein yields (see figure 2. Below), whereas cobalt is preferred for higher purity preparations.

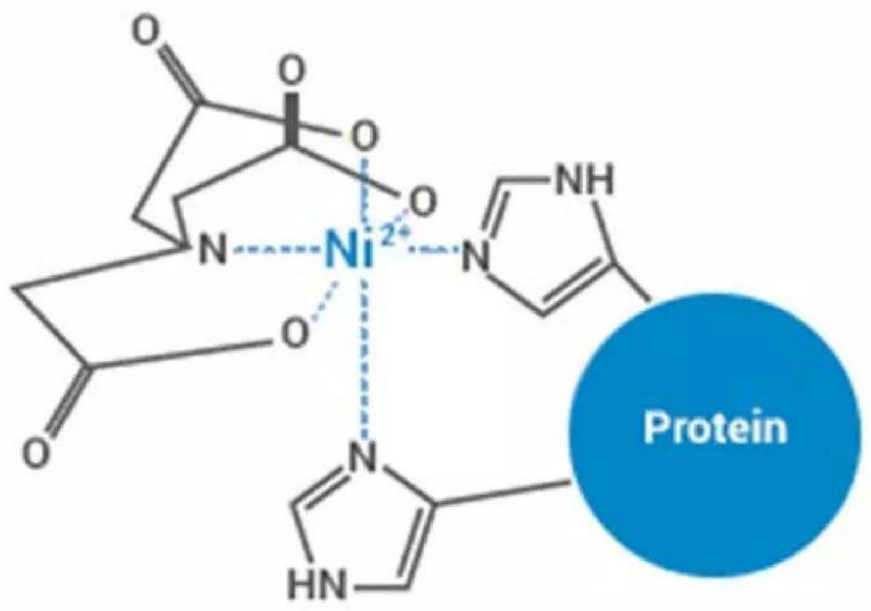

Figure 2. Selective Binding of Nickel (Ni^{2+}) Chelated Resin to His Tag Recombinant Protein.

DETECTION OF HIS TAG PROTEINS

Epitope tagging is a common method employed for the identification and detection of proteins. In a similar manner, the His Tag also acts as a specific site for a binding partner, such as an antibody with high specificity, so allowing easy detection of the recombinant protein of interest. Downstream applications such as SDS PAGE and Western blot can subsequently detect and quantify the His Tag protein following purification to determine yield, especially where anti His Tag antibodies are available. Other uses include protein localization, protein to protein interaction and immunoprecipitation studies.

EXPEDEON TECHNOLOGIES FOR EASY VALIDATION OF HIS TAG PROTEIN EXPRESSION:

Traditional methods to assess the success of protein expression and purification could be tedious and time consuming. Expedeon's His Tag Check&Go! kit is a quick and easy competitive immunochromatography test developed to allow scientists to verify and monitor successful expression of His tagged recombinant proteins, prior to performing other downstream applications, in one easy step.

His Tag Check&Go! is a simple qualitative immunochromatography test. The key component of the kit is a nitrocellulose membrane containing immobilized His Tag protein on the 'Test line' (T line) and an ULFA tagged protein on the 'Control line' (C line). A 40nm InnovaCoat®-GOLD anti His Tag antibody and 40nm InnovaCoat®-GOLD anti ULFA tag conjugate are used for detection.

Benefits of His Tag Check&Go! include:

- Quick yes / no answer during expression and purification processes
- Results in less than 15 minutes!

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- Compatible with cell culture media and lysate
- Easy to use
- Cheaper than gel electrophoresis / Western blot
- Small sample volume required: 80ul diluted* sample / strip
- Stable C line signal intensity: Independent from His tagged protein levels
- Compatible with AmintraTM CoHis, NiHis and Ni-NTA resins.

* Subject to protein yield

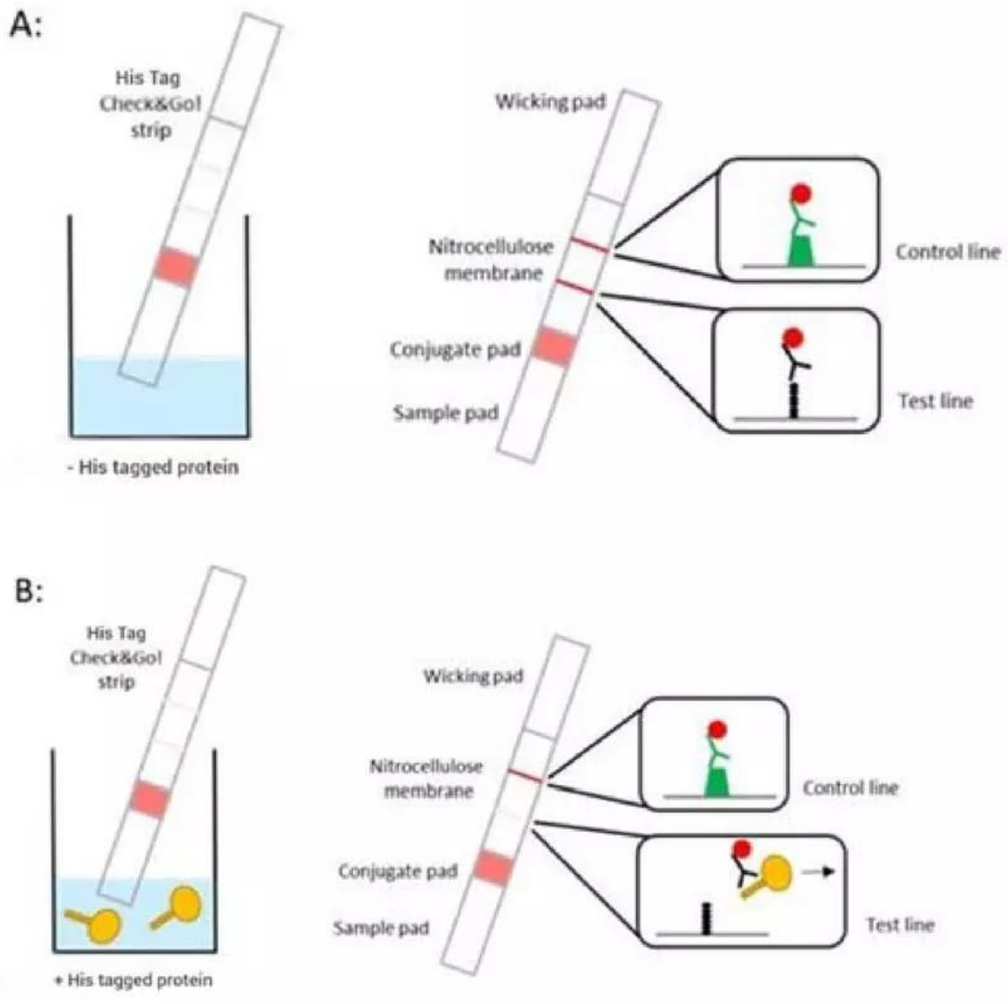

Figure 3. Easy Visualization of Results for His Tag Check&Go! Expression Validation Kit.

A) If the sample does not contain any His tagged protein, the T line will appear as a strong red line (See above: -His tagged protein)

B) If the sample does contain His tagged protein, the T line will not be visible (or it will appear as a pale red line) as it competes with the binding of the InnovaCoat® -GOLD anti His Tag antibody conjugate to the antigen immobilized on the T line (See above: +His tagged protein).

NB: The C line is assay independent and should always appear as a strong red line – If it is not visible, the test is invalid and should be repeated.

EXPEDEON RELATED PRODUCTS FOR HIS TAG PROTEIN PURIFICATION:

Expedeon also offers a range of products to enable the purification of His Tag recombinant proteins. These include our Amintra™ NTA Resins, Amintra™ Nickel Magnetic Beads and AminTRAP HIS Prepacked Columns:

Amintra™ Ni-NTA affinity resin
Amintra™ Cobalt NTA Resin
Amintra™ Nickel Magnetic for His tagged proteins
AminTRAP HIS Prepacked Columns

Find out more here.

References

- Darain, F., Ban, C., & Shim, Y.-B. Development of a new and simple method for the detection of histidine tagged proteins. *Biosensors and Bioelectronics*. 2004:**20**(4);857–863. doi:10.1016/j.bios.2004.03.028.
- Ravikumar, R., Chen, L. H., Jayaraman, P., et al. Chitosan-nickel film based interferometric optical fiber sensor for label free detection of histidine tagged proteins. *Biosensors and Bioelectronics*. 2018:**99**, 578–585. doi:10.1016/j.bios.2017.08.012.
- Uchinomiya, S., Nonaka, H., Wakayama, S., et al. In-cell covalent labeling of reactive His Tag fused proteins. *Chemical Communications*. 2013:**49**(44); 5022. doi:10.1039/c3cc41979g.