

## Purification of His-tagged proteins from inclusion bodies using QuickPick™ IMAC

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### ABSTRACT

Over-expression of recombinant proteins frequently leads to the production of inclusion bodies, which are insoluble aggregates of unfolded protein. However, inclusion bodies can easily be solubilized and purified under strongly denaturing conditions.

Standard protein purification/separation methods rely on conventional liquid chromatography strategies based on chromatographic or spin columns. However, these methods are time-consuming and complex.

QuickPick™ IMAC (Immobilized Metal Affinity Chromatography) kit can be used to carry out the purification of His-tagged proteins quickly and easily. The QuickPick methods are especially convenient for small sample volumes with emphasis on fast and material-conserving analysis.

### INTRODUCTION

Genetically engineered proteins containing a histidine tail (His-tag) are widely used in both protein expression and protein function studies. In many cases and in several host systems, these recombinant proteins accumulate in cells as insoluble aggregates, so-called inclusion bodies. The proteins expressed as inclusion bodies are mostly inactive and denatured. The formation of inclusion bodies is a frequent consequence of high-level protein production in the cytoplasm. It is not possible to generalize or predict which proteins are produced as inclusion bodies.

Production of recombinant proteins as inclusion bodies has several advantages:

- The recombinant protein deposited as inclusion bodies can be 50% or more of the total cellular protein.
- Inclusion bodies often contain almost exclusively the over-expressed protein.
- In the form of inclusion bodies the protein is protected from proteolytic degradation resulting in higher yield.
- Expression as inclusion bodies will protect the cell against the toxicity of the recombinant protein since inclusion bodies have no biological activity.
- Inclusion bodies can be accumulated in the cytoplasm to a much higher level than when produced in soluble form.

The PickPen™ technology using IMAC magnetic particles has several benefits in comparison to other methods, some of the most significant being speed and ease of use. The time needed for a single purification is only 5 minutes.

The method is optimized for small sample volumes. However, with samples containing a low protein concentration volumes up to 1 ml can be used. In fact, PickPen technology also serves as a method to concentrate the target protein and thereby facilitate, for example, the detection of proteins expressed in low levels.

### MATERIALS AND METHODS

QuickPick™ IMAC kit was modified to obtain denaturing conditions as follows:

**IMAC Wash Buffer 1** was prepared by dissolving 1,6 g urea in 1700 µl of IMAC 2<sup>x</sup>Wash Stock Buffer and adding H<sub>2</sub>O to a final volume of 3400 µl. Final urea concentration is 8 M.

**IMAC Wash Buffer 2** was prepared by dissolving 1,2 g urea in 1700 µl of IMAC 2<sup>x</sup>Wash Stock Buffer, adding 135 µl of IMAC Imidazole Buffer, 500 mM, mixing well and adding H<sub>2</sub>O to a final volume of 3400 µl. Final urea and imidazole concentrations are 6 M and 20 mM, respectively.

**IMAC Elution Buffer** was prepared by dissolving 290 mg urea in 800  $\mu$ l of IMAC Elution Buffer. Final urea and imidazole concentrations are 6 M and 240 mM, respectively.

Inclusion bodies were produced by expressing the His-tagged Btk SH<sub>2</sub>(H362Q) protein variant in *E. coli* BL21(DE3) strain. 1.5 ml of the *E. coli* culture was centrifuged and the cell pellet was suspended in 300  $\mu$ l of IMAC Wash Buffer 1 (containing 8 M urea). The suspension was sonicated for 1 min (5 s pulses and 9,9 s intervals) and after sonication the suspension was incubated for 5 minutes at room temperature with mild shaking (15 rpm) to solubilize the inclusion bodies. The suspension was centrifuged for 5 min 20 000 x g at room temperature and the clear supernatant was used as a sample.

For certain proteins the 5 minute solubilization time might be too short. However, with the Btk SH<sub>2</sub>(H362Q) protein variant the results were almost as good using 5 minutes solubilization as with 8 hour solubilization. Also, for some proteins 6 M guanidium hydrochloride might be needed instead of 8 M urea, but guanidium hydrochloride should be used only in IMAC Wash Buffer 1 and in both cases urea should be used in IMAC Wash Buffer 2 and IMAC Elution Buffer.

As an example of the purification of recombinant protein from inclusion bodies, the procedure using the QuickPick IMAC kit is described (Fig. 1a-f). The IMAC magnetic particles were first suspended in the IMAC Regeneration Buffer (a) and then transferred into IMAC Wash Buffer 1 (containing 8 M urea) (b). The equilibrated particles were incubated for 2 min with the sample (c), washed once in IMAC Wash Buffer 2 (containing 6 M urea) (d) and to conclude the bound proteins were eluted out by incubating the particles in 25  $\mu$ l of the IMAC Elution Buffer (containing 6 M urea) (e) for 1 min.

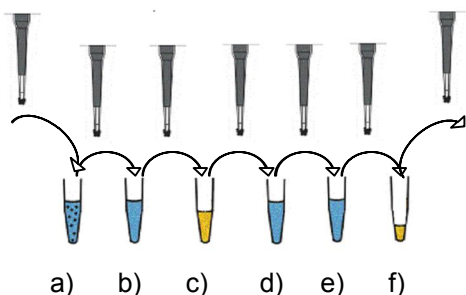


Fig. 1

The QuickPick IMAC kit reagents from Bio-Nobile™ were used in the purification.

**RESULTS**

To demonstrate the purification properties of IMAC magnetic particles with PickPen technology, the solubilized inclusion body supernatant and the final elution solution were analyzed in SDS-PAGE (Fig. 2). The gel picture shows the binding and elution behavior of proteins during the procedure. The yield of the Btk SH<sub>2</sub>(H362Q) protein variant has been shown to be approximately 20  $\mu$ g per preparation using the QuickPick IMAC kit.

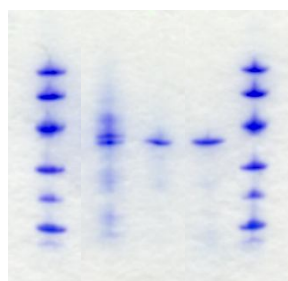


Fig. 2 SDS-PAGE gel picture

- Lane 1: LMW-marker
- Lane 2: 10  $\mu$ l of solubilized supernatant sample, (before magnetic particle treatment)
- Lane 3: 10  $\mu$ l of elution buffer (5 min solubilization)
- Lane 4: 10  $\mu$ l of elution buffer (8 hour solubilization)
- Lane 5: LMW marker

**CONCLUSIONS**

Proteins expressed as inclusion bodies require solubilization before purification. Urea is added to the QuickPick IMAC kit buffers to provide a simple protocol for solubilizing proteins before carrying out the purification. The QuickPick IMAC method together with PickPen transfer technology gives a fast and cost-effective means of purifying His-tagged proteins whether they are present in inclusion bodies or not.