IMAC resins – should Ni always be the metal of choice?

In the purification of a protein, the performance of the resin in terms of duration of the overall process/number of steps required can be just as important as the purity of the resulting protein. For clients purifying proteins on a larger scale or with a large throughput, the number of process steps therefore becomes an important economic consideration. Conventional resins usually provide adequate results for the majority of proteins being purified. However, they will not necessarily provide optimal results in terms of purity and yield in every situation.

The University of Manchester Protein Expression Facility purifies more than 500 different proteins per year. The facility was originally set up to provide an 'in-house' protein purification service for researchers at the University of Manchester Interdisciplinary Biocentre (MIB). Clients now include not only academics but also pharmaceutical companies for which purification processes with improved efficiency could offer a significant economic advantage.

Proteins obtained from a variety of expression systems (bacteria, yeast and insect cells) are generally purified in conjunction with pre-validated protein purification resins. Occasionally an opportunity arises to test resins from other manufacturers with a view to maximising the efficiency of the processes used and ultimately try and reduce costs. In one such test carried out, Agarose Bead Technologies' IMAC IDA resins; Ni2+ (High and Low loaded), Co2+ (High loaded) and Zn2+ (Low loaded) supplied by Web Scientific, were compared against other resins routinely used in the lab for their ability to bind and purify a 45 KDa protein. Resins were tested 1) for their ability to bind target protein under specified binding conditions (50mM Sodium Phosphate, pH7.0 for ABT resins; 25mM Tris-HCL, pH8.0 for normal lab resins) and 2) the purity of the eluted protein.

Figure 1. compares the quantity and purity of target protein eluted from each resin. The capacity of ABT resins and purity of target protein obtained from them was as good if not slightly better than that obtained from the Ni NTA and IDA resins normally used in the lab. The results also showed that, compared to the other resins tested, ABT's Zn2+ Low loaded resin was capable of producing protein of a higher purity after an equivalent number of wash steps (x2 for each resin). Since 5-6 wash steps are typically used to produce protein of a similar level of purity using the usual Ni-NTA and IDA lab resins, these results imply the possibility for significant time and cost savings when using ABTs Zn2+ resin in routine protein purifications. With an increase in the number of researchers at the MIB requiring crystallography grade protein purity, these results also imply the potential of ABTs Zn2+ resin in producing protein of a superior purity if a greater number of wash steps were applied.

It is worth noting here the clear advantage offered by having a range of IMAC resins to choose from in the design of protein purification protocols. The availability of resins charged with different divalent metal ions and to different densities allows the end user to 'fine-tune' the purification process and much more accurately home in on the target protein.

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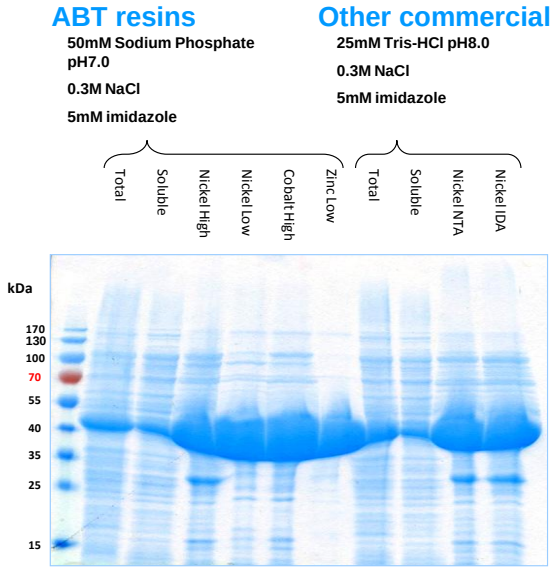

Fig 1. Cell pellet (Protein X) resuspended in 30ml selected buffer + 1%TX100 + Protease Inhibitor Cocktail (1/1000), sonicated seven times 20sec on/off on ice and clarified at 17Krpm 20min 4ºC. 500ul of soluble fraction incubated with prewashed resins for 2hours whilst mixing at 4ºC. The beads were washed 2x500ul with buffer for 5min each and protein boiled. Total, Soluble & Bound bead fractions were analysed by **12% SDS PAGE Bio-Safe stain**