TALON™ Resin Offers 6xHis Protein Purification Under Native Conditions Using β-Mercaptoethanol as a Reducing Agent

Cindy Leo, Paul Nelson, Ph.D., Steve Kain, Ph.D. & Teresa Yang, Ph.D.
Cell Biology and Nucleic Acids Chemistry Groups
CLONTECH Laboratories, Inc.

Abstract

TALON™ Metal Affinity Resin is a non-nickel, immobilized metal affinity chromatography (IMAC) resin introduced last year by CLONTECH (1). TALON is designed for durability and versatility in purifying 6xHis-tagged proteins and to overcome disadvantages of other commercially available IMAC resins. These disadvantages include metal leaching, limited resolving power, and incompatibility with reducing agents (1, 2). Here we show TALON's performance under native conditions using up to 30 mM β-mercaptoethanol (βME) as a reducing agent.

Many proteins require free sulfhydryl groups to maintain their biological activities. Reducing agents such as βME in the purification buffers can not only maintain the biological activities, but also increase the yields of purified proteins. However, most IMAC resins are incompatible with reducing agents such as βME (3, 4).

High protein purity and no metal leaching with βME

To analyze the purity of 6xHis proteins purified using TALON under native conditions with βME as a reducing agent, mouse DHFR was expressed in *E. coli* and purified with TALON resin. Figure 1 illustrates the exceptional purity of 6xHis dihydrofolate reductase (DHFR) obtained using TALON with up to 30 mM βME in the native purification buffer. Moreover, the lack of a predominant band at 19.5 kDa in the flowthrough lanes indicates that virtually no loss of metal (metal leaching) occurred during protein purification using TALON under these conditions.

TALON uses a special divalent metal ion to affinity bind 6xHis proteins. This metal ion is strongly anchored to sepharose beads by a polydentate metal chelator that is ideal for binding octahedral metals. This design prevents metal leaching, offering a significant advantage of TALON over other IDA-based (iminodiacetic acid) IMAC resins, which may lose their metal during purification because the metal is not firmly bound (1, 2).

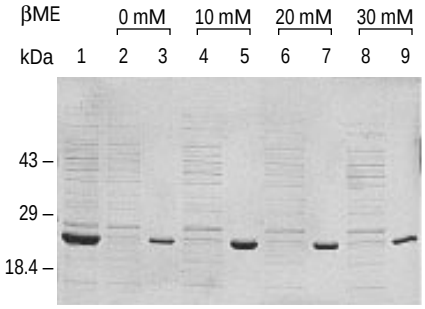

Figure 1. TALON 6xHis DHFR purification under native conditions with increasing concentrations of βME. N-terminal 6xHis-tagged mouse DHFR (19.5 kDa) was expressed in *E. coli*. 25-ml cell cultures were disrupted in 2 ml of sonication buffer (pH 8.0) by freezing and thawing. 2.66 ml of clarified lysate (4 mg/ml total protein) was applied to 200 µl of TALON per gravity flow column, pre-equilibrated with the sonication buffer. The 6xHis protein/resin complexes were washed 3X with sonication buffer, pH 8.0. All bound protein was eluted with 600 µl of 100 mM EDTA. The indicated concentrations of βME were included in the buffer. Samples were electrophoresed on a 12% polyacrylamide/SDS gel, and the gel was stained with Coomassie blue. Lane 1: 20 µl of cell lysate. Lanes 2, 4, 6, & 8: 20 µl of flowthrough. Lanes 3, 5, 7, & 9: 5 µl of eluant.

Better yields using βME with TALON than with Ni-NTA

We also compared the yields of 6xHis proteins purified using TALON with yields using the leading Ni-NTA resin under native conditions with up to 30 mM βME in the buffer. Figure 2 shows that the yield of purified 6xHis DHFR was significantly higher at 10, 20, and 30 mM βME using TALON compared to using Ni-NTA-based resin. The data also indicate that with TALON, low concentrations of βME in the buffer can actually increase 6xHis protein yields. Furthermore, up to 30 mM βME can be used with TALON without reducing the purity of 6xHis protein obtained (Figure 1).

These data show TALON's excellent performance for native protein purification using up to 30 mM βME as a reducing agent in the purification buffer. The high yields and purity of 6xHis proteins purified under native conditions enable researchers to quickly and easily obtain sufficient quantity and quality of recombinant proteins of interest. These can be used for studying the biological activities or for any other applications that require proteins to be in their native conformations. ■

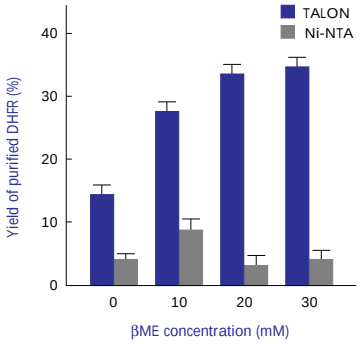

Figure 2. Yields of recombinant 6xHis DHFR from cell extracts purified by TALON versus Nickel-NTA in the presence of βME. N-terminal 6xHis DHFR was expressed and purified under native conditions as described in Figure 1. Protein concentrations were determined by Bradford assay (5). Yields are expressed as a percentage of total protein in the cell lysate.

References

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