Fast, Easy His-Tagged Protein Purification

HisTALON™ Cartridges (1 ml) offer rapid, high-performance automated or syringe-based protein purification

- **Maximizes yield of biologically active protein**
- **Automated or syringe-based protocols**
- **Superior purity & high yields with TALON® Superflow Resin**
- **Over 2,400 peer-reviewed publications using TALON resin**

Figure 1. The HisTALON Cartridge (1 ml) provides a highly efficient and specific method for purifying his-tagged proteins.

The ability to preserve the biological activity of a target protein and improve its yield during sample preparation and purification procedures depends largely on the conditions under which these procedures are carried out and the speed at which they can be performed. Factors such as excessively low or high pH and the presence of proteases and additives can decrease yield. The negative impact of either factor can be diminished or eliminated by shortening these procedures. Thus, a quick, efficient sample preparation procedure and a speedy, quantitative capture-and-release follow-up step, combined with an easy-to-use system, will help maximize the yield of active target protein.

Faster protein purification can be achieved with a resin that functions at higher flow rates, such as our highly-regarded **TALON Superflow Resin,** which can withstand flow rates of 5–20 cm per min. This resin combines Superflow 6, a rigid, highly porous agarose derivative, with TALON, a highly selective immobilized metal ion affinity chromatography (IMAC) ligand (1–6). TALON is a tetradentate chelator charged with cobalt and specific for his-tagged proteins. We are expanding on the success of TALON Superflow Resin with our ready-to-use **HisTALON Cartridges** (Figure 1). These cartridges are prepacked with the resin, and designed for efficient purification of his-tagged proteins from a total soluble protein extract of bacterial, mammalian, or baculovirus-infected cells.

Table I: Comparison of HisTALON Cartridge (1 ml) Protein Yield & Activity to that of Competitor Cartridges[1]

	Starting Sample		Flowthrough		Wash		Eluate	
Vendor	Protein (mg)	AcGFP1 (RFU)	Protein (mg)	AcGFP1 (RFU)	Protein (mg)	AcGFP1 (RFU)	Protein (mg)	AcGFP1 (RFU)
Clontech	22.3	202,167	21.1	9,363	0.1	292	1.1	198,114
Vendor G[2]	12.7	82,845	12.2	4,980	0.1	215	0.6	81,111
Vendor Q[2]	12.4	88,300	11.3	4,560	0.2	3,600	0.7	78,320
Vendor P[3]	20.6	201,174	18.6	10,304	0.14	653	1.311	193,214

1 Extraction and chromatography were performed according to the respective vendor's recommendations (see the Figure 2 caption for the Clontech sample extraction procedure).

2 Extraction in presence of lysozyme.

3 Extraction with the product-specific recommended extraction buffer.

Maximize Yield of Active Protein

HisTALON Cartridges are designed to maximize your yield of biologically active protein. The stable chelation of the Co^{2+} ion, combined with the specificity of the TALON reactive core, deliver unmatched purity (activity relative to amount of protein) when compared to three competitor cartridges (Table I). Up to 20 mg of his-tagged AcGFP1 can be adsorbed on one HisTALON cartridge.

Highly Reproducible & Rapid Results

Figure 2 presents the chromatography data from three consecutive runs on a HisTALON cartridge, using the HisTALON Buffer Set for protein extraction and purification. High reproducibility was observed between runs, with differences of less than 5% in both protein content and fluorescence signal (data not shown). The entire process, including extraction and purification, required less than 1 hour.

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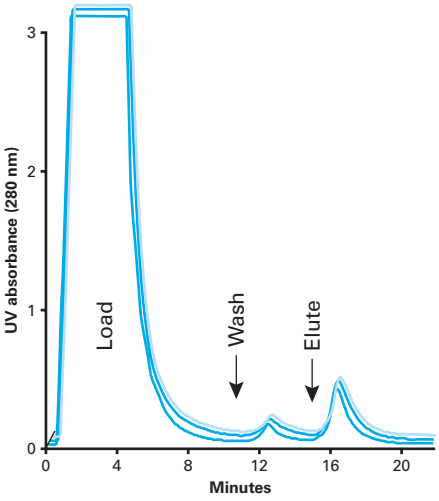

Figure 2. HisTALON Cartridges provide highly reproducible and rapid his-tagged protein purification. Protein extraction and chromatography were performed according to the User Manual, with 0.4 g pellets of *E. coli* cells expressing his-tagged AcGFP1. These cells were extracted in the recommended volumes of extraction buffer and centrifuged at 10,000 x g for 20 min, at 4°C. For each run, extract from 250 mg of cells was consequently applied to the cartridge using the recommended loading buffer and flow rate, on an FPLC system from GE Healthcare (used for all the purifications described in this article). After the wash and elution steps were completed, all fractions, as well as the original samples, were analyzed for protein content (7) and relative fluorescence (from active AcGFP1) on a 96-well fluorescence spectrophotometer. A high level of reproducibility was observed between runs, with differences of less than 5% in both protein content and fluorescent signal (data not shown). The entire process, including extraction and purification, was completed in less than 1 hour.

Easy Automated or Syringe-Based Purification

HisTALON Cartridges, in an optional combination with an optimized buffer set, provide efficient and seamless integration of upstream sample preparation, with quick and selective purification on automated systems such as ÄKTA™ FPLC™ and other medium-pressure, automated protein purification systems. The cartridges and buffers are available separately as the **HisTALON Cartridge** (providing 5 x 1 ml cartridges) and the **HisTALON Buffer Set,** respectively—or in combination as the **HisTALON Cartridge Purification Kit.** The cartridge may also be adapted for manual, syringe-based protein purification with appropriate couplings, such as Luer Lock Syringe Fittings (GE Healthcare, Cat. No. 18-1112-51) and the original M6 FPLC fittings (GE Healthcare, Cat. Nos. 18-1112-58 & 18-1112-57). HisTALON Cartridges enable fast, easy, and reproducible chromatographic separations and can be regenerated for multiple uses. However, we recommend that you reuse a cartridge only to purify different batches of the same protein, and utilize the complete regeneration method described in the User Manual.

Highly Selective Resin Provides Superior Purity

The HisTALON Cartridge yielded superior results when chromatography fractions were compared by SDS-PAGE to those of a competitor (Figure 3). A contaminating high molecular weight band was detected in the Vendor P eluate, but not in the HisTALON eluate.

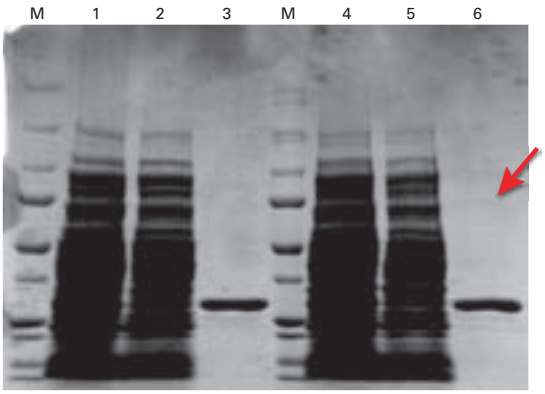

Figure 3. HisTALON Cartridges yield higher purity than a competitor. Fractions from the purification of his-tagged AcGFP1 on respective HisTALON and Vendor P cartridges were compared via electrophoresis on a 4–15% SDS polyacrylamide gel (Bio-Rad Laboratories) stained with Coomassie Blue. Lanes M: Bio-Rad® Precision Plus Protein™ All Blue Standards. Lane 1: HisTALON extract. Lane 2: HisTALON flowthrough. Lane 3: HisTALON eluate. Lane 4: Vendor P extract. Lane 5: Vendor P flowthrough. Lane 6: Vendor P eluate. A contaminating high molecular weight band appeared in Lane 6 (Vendor P eluate), but not in Lane 3 (HisTALON eluate), indicating that the HisTALON eluate was of higher purity—consistent with the data in Table I.

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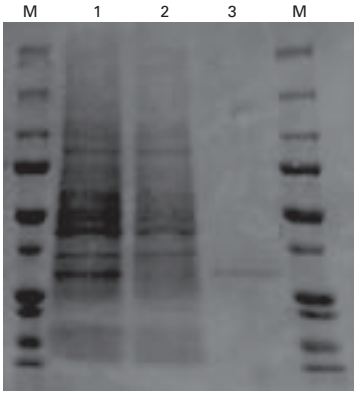

Figure 4. HisTALON Cartridges effectively purify mammalian samples. A 200 mg HEK 293 cell pellet sample was extracted with our TALON xTractor Buffer. The extract, spiked with 25 µg of AcGFP1 and containing more than 10 mg of mammalian proteins from the HEK 293 cells, was loaded on a HisTALON cartridge. Fractions were analyzed via electrophoresis on a 4–15% SDS polyacrylamide gel and visualized with a silver staining kit (both from Bio-Rad Laboratories). Lanes M: Bio-Rad Precision Plus Protein All Blue Standards. Lane 1: HEK 293 extract spiked with 25 µg of AcGFP1. Lane 2: flow-through. Lane 3: eluate. The single band in Lane 3 demonstrates the high purity of the eluate.

Our cobalt-based TALON resin is the tool of choice for numerous researchers (in over 2,400 peer-reviewed publications), to help them obtain the purest possible target protein. They chose TALON because of its high specificity and selectivity: TALON resin will adsorb your his-tagged protein of interest, while adsorbing insignificant amounts of impurities. In many cases, when a protein is produced in trace amounts, such an approach is the only viable alternative to multistep purification processes that yield very low recoveries, require much time to develop, and present the risk of purifying proteins that are degradation and or modification variants of the target protein (proteolytic, oxidation, or denatured products).

Effective Purification of Mammalian Proteins

In order to demonstrate the utility of our HisTALON Cartridges for mammalian sample applications, we extracted a HEK 293 cell pellet, spiked with 25 µg of AcGFP1, with our TALON xTractor Buffer (supplied with the HisTALON Buffer Set and HisTALON Cartridge Purification Kit) and purified the extract on a HisTALON cartridge. The entire fluorescence signal was recovered in the eluate and none was detected in the flowthrough (data not shown). When chromatography fractions were analyzed by SDS-PAGE (Figure 4), the eluted material appeared to be extremely pure, despite the fact that the original 25 µg of AcGFP1 were spiked in more than 10 mg of mammalian proteins from the HEK 293 cell extract.

HisTALON Cartridges provide the unsurpassed performance of our TALON Superflow Resin in a convenient prepacked format. When used in combination with our HisTALON Buffer Set, researchers can save time, ensure efficient sample extraction while preserving the integrity of target proteins, and achieve the highest purity and yields.

Product	Size	Cat. No.
HisTALON Cartridge	5 x 1 ml	635650
HisTALON Buffer Set	20 purifications	635651
HisTALON Cartridge Purification Kit[1]	5 x 1 ml	635649

NEW!

1 The HisTALON Cartridge Purification Kit combines the HisTALON Cartridge (containing 5 x 1 ml cartridges) and the HisTALON Buffer Set.

Notice to Purchaser

Please see the TALON® Purification Products licensing statement on page 40.

References

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