Profinity™ IMAC Resin: An Optimal Support for His-Tagged Protein Purification

Tech Note

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Protein Purification

Bulletin 5456

Introduction

Immobilized metal affinity chromatography (IMAC) is a powerful technique that can be used for the efficient purification of recombinant histidine (His)-tagged proteins from a variety of expression systems.

The synthesis of IMAC resins begins with the derivatization of an appropriate wide-pore base resin with metal-chelating groups, such as iminodiacetic acid (IDA) or nitrilotriacetic acid (NTA). Specific transition metal ions, usually Cu^{2+}, Ni^{2+}, Co^{2+}, Ca^{2+}, or Zn^{2+}, then bind to the chelating groups to form an immobilized metal affinity support. IMAC resins typically exhibit high affinity and metal-dependent selectivity for His-tagged proteins as well as naturally occurring proteins that are rich in histidine, cysteine, aspartatic acid, or glutamic acid residues.

Profinity IMAC Resin is based on Bio-Rad's patented† UNOsphere™ technology. Profinity IMAC beads are 60 µm particles derivatized with IDA. This resin exhibits mechanical strength and excellent pressure-flow properties that allow protein separation at high flow rates without compromising binding capacity, recovery, or final purity of target proteins. Its ligand density has also been optimized to reduce nonspecific binding. Profinity IMAC Resin has an open pore structure, making it particularly useful for purifying proteins over a wide molecular size range, especially for purification of larger proteins.

Methods

Chromatography Systems and Reagents

Liquid chromatography system — The BioLogic DuoFlow™ System or the BioLogic DuoFlow Maximizer™ System, which provides a flow rate up to 80 ml/min, were used for all pressure-flow and dynamic binding studies. The accuracy of the pressure sensor was checked using an external pressure gauge (PG2000 Digital Pressure Gauge, PSI-Tronix, Inc.). High-pressure FEP tubing (3.2 mm OD, 1.6 mm ID) was used to reduce the system pressure.

Resins — Profinity IMAC Resin and IMAC resins from three other commercial suppliers were used. These included both precharged and uncharged resins with various ligands (Table 1). Resins that had a significant market share at the time of testing were selected for comparison.

† US patent 6,423,666.

Table 1. Resins and protein samples used to evaluate His-tagged protein purification.

Materials Used	Properties
Protein samples	*E. coli* lysate containing one of the following: ▪ 32 kD His-tagged protein from *Anabaena* sp. strain PCC 7120* ▪ $(His)_6$-GFP ▪ 250 kD His-tagged recombinant protein ▪ 75 kD His-tagged recombinant protein Purified His-tagged NIF-3
Resins	Profinity IMAC Resin (IDA resin) IMAC resins from supplier A ▪ Resin 1, uncharged agarose-based resin (IDA ligand), charged with Ni^{2+} before use ▪ Resin 2, Ni-charged high-binding-capacity agarose-based resin (IDA ligand) ▪ Resin 3, newer Ni-charged high-binding-capacity agarose-based resin (IDA ligand) IMAC resin from supplier B (NTA ligand) IMAC resin from supplier C (N-carboxymethyl aspartic acid ligand)

* Courtesy of Dr Ray Stevens, University of California, Berkeley, CA, USA.

Protein samples — The samples used were selected based on their size, solubility, and availability, and are summarized in Table 1.

Columns — 1.1 x 30 cm Amicon Columns were used for pressure-flow, maximum operating pressure, and shrink/swell studies. Bio-Scale™ MT2 Columns and GE Healthcare HR 5/5 Columns were used for studies of dynamic protein binding to resins.

Batch binding studies used Micro Bio-Spin™ Spin Columns packed with IMAC resins to study their selectivity, with elution at 1,000 x g in a benchtop centrifuge.

Buffers — Performance of IMAC resins was compared using buffers commonly used for native and denaturing chromatographic protein purification as detailed for individual experiments. Buffers used to elute bound His-tagged proteins contained imidazole, which selectively competes with His for metal binding sites.

An SBS Model DV-100 Viscometer (Stony Brook Scientific, Ltd.) was used to determine the viscosity of buffers or other solutions used in tests of column performance.

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Evaluation of Chromatographic Properties

Column packing — All the IMAC resins were converted to a 50% (v/v) slurry in 20 mM sodium phosphate. The columns were packed to a bed height of approximately 20 cm under a flow of 20 mM sodium phosphate up to 43 psi (3 bar). The column adaptor was manually pressed down after flow-packing to further compress the column. All columns were initially packed with the 20 mM sodium phosphate; for tests using other buffers, the columns were equilibrated in more than 3 column volumes (CV) of these buffers before testing.

Pressure testing — All the pressure data in this report were calculated proportionally to the values for a column with a 20 cm bed height. We studied three resin properties that are relevant for chromatography applications by performing pressure-flow, net maximum operating pressure, and shrink/swell tests.

Pressure-flow tests measure the relationship between the flow rate and the column pressure for different buffers within normal operating conditions; that is, pressure ≤43 psi and flow rate ≤1,200 cm/hr. Buffers or other solutions of different viscosities were used. The pressures at each flow rate were recorded until the pressure exceeded 43 psi.

The net maximum operating pressure is assessed by gradually increasing the flow rate, and is defined as the point where the pressure shows a sudden change and the column bed is seriously compressed, damaged, or destroyed. Pressures at each flow rate were recorded until the pressure showed a sudden change and exceeded 500 psi. The net maximum operating pressure was defined as the point of intersection of the two tangents to the pressure-flow curve.

Shrink/swell tests measure the changes of column bed height in different buffers with increasing flow rate. The column bed height and the pressures at each flow rate were recorded.

Other physical characteristics — Additional studies were performed to evaluate other characteristics of Profinity IMAC Resin, including its stability to repeated cycling, mass transfer kinetics, and dynamic binding capacity.

To evaluate cycling stability, a 1 ml HR 5/5 column packed with Profinity IMAC was subjected to 201 cycles of simulated chromatography.

To evaluate mass transfer kinetics, crude *E. coli* lysate containing a recombinant 75 kD His-tagged protein was applied to a 1 ml column and eluted in a series of loading-equilibration (washing)-elution cycles at various flow rates.

Dynamic binding capacity was determined by continuously loading a sample of known concentration onto a column and monitoring the protein in the flowthrough. When the quantity of the protein in the flowthrough exceeded 10% ($Q_{10\%}$ or 10% breakthrough), sample application stopped, and the amount of applied protein was calculated.

Electrophoresis and Protein Quantitation

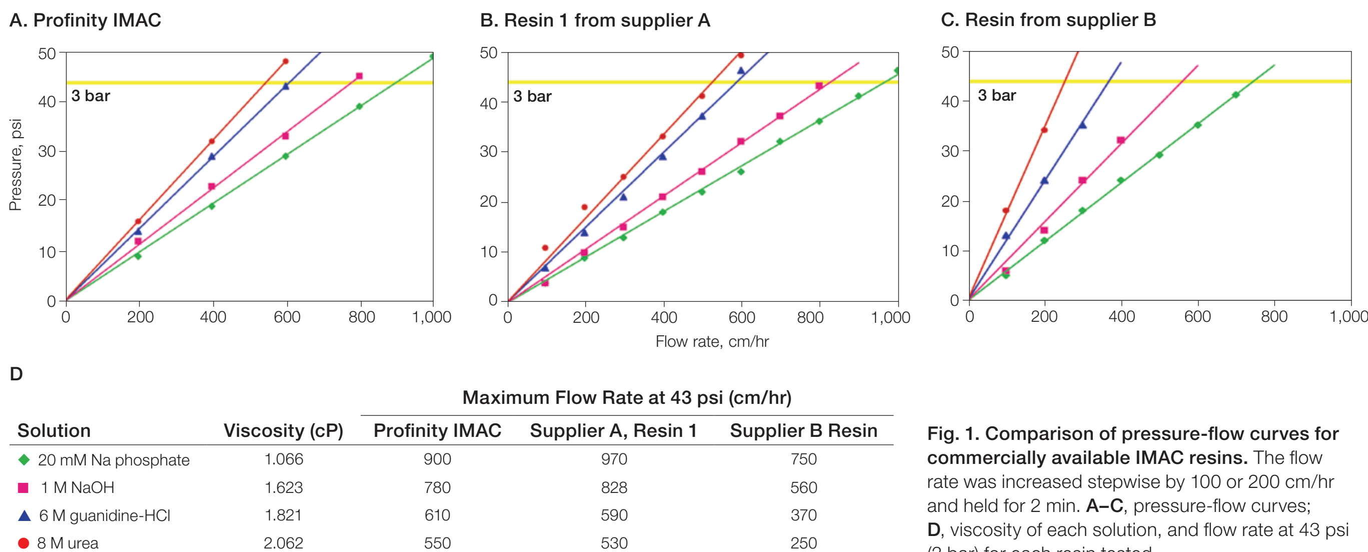
The dynamic binding capacities of Profinity IMAC and other IMAC adsorbents for various His-tagged proteins and the resulting yield and purity of the target protein were evaluated by analyzing chromatographic fractions by UV/visible spectroscopy and SDS-PAGE. Normalized protein elution was used for SDS-PAGE analysis, which was performed using Criterion™ Tris-HCl Gels. Gels stained with Coomassie Blue were analyzed using Quantity One® Software to determine purity of individual protein bands.

Results and Discussion

Physical Performance of IMAC Resins

Columns packed to a height of 20 cm at up to 43 bar were further compressed by pressing the adaptor down after flow-packing. This further compressed the Profinity IMAC column bed 10 mm (5%). In contrast, the IMAC resin 1 from supplier A and the IMAC resin from supplier B were only compressed an additional 1 mm.

The pressure-flow experiments showed good reproducibility; that is, similar results for different runs on the same column, and on different columns using the same resin (Figure 1). The overall experimental error was ±5 psi. Profinity IMAC pressure-flow curves showed desirable shallow slopes, as did the curves for resin 1 from supplier A, even though the latter has a much



Solution	Viscosity (cP)	Maximum Flow Rate at 43 psi (cm/hr)		
		Profinity IMAC	Supplier A, Resin 1	Supplier B Resin
◆ 20 mM Na phosphate	1.066	900	970	750
■ 1 M NaOH	1.623	780	828	560
▲ 6 M guanidine-HCl	1.821	610	590	370
● 8 M urea	2.062	550	530	250

Fig. 1. Comparison of pressure-flow curves for commercially available IMAC resins. The flow rate was increased stepwise by 100 or 200 cm/hr and held for 2 min. **A–C**, pressure-flow curves; **D**, viscosity of each solution, and flow rate at 43 psi (3 bar) for each resin tested.

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larger particle size (90 µm, compared to 60 µm). IMAC resin from supplier B pressure-flow curves showed much steeper slopes. Profinity IMAC showed high linear flow rates below 43 bar, which means that separations can be performed even with comparatively high-viscosity buffers at reasonable flow rates. These values are similar to those of resin 1 from supplier A and much better than those for IMAC resin from supplier B.

The results of the maximum net operating pressure testing are shown in Figure 2. The net maximum operating pressure for Profinity IMAC was >100 psi at a flow rate of about 2,400 cm/hr. This value was much higher than those found for the IMAC resins from suppliers A and B. Resin 1 from supplier A and IMAC resin from supplier B are both agar-based hydrogel-type materials with net maximum operating pressures <50 psi. Profinity IMAC Resin is composed of highly crosslinked polymeric particles; the nonlinear portion of the pressure-flow curve for this resin was in the higher-pressure region, indicating that it is capable of stable operation at higher pressures (43–100 psi) than the other resins.

The shrink/swell testing results for Profinity IMAC are shown in Figure 3. Ethanol-water mixtures caused the most shrinking on the column bed. In contrast, 20 mM sodium phosphate, deionized water, and 1 M NaCl did not cause significant shrinking. The tested solutions in increasing order of shrinkage effect were: 1 M NaOH, 6 M guanidine-HCl, 8 M urea, 20% ethanol, and 70% ethanol.

After repeated cycling, Profinity IMAC Resin delivered consistent and reproducible separation (Figure 4), a desirable feature for routine and especially process chromatography applications.

The mass transfer kinetics results with Profinity IMAC showed that, regardless of flow rate used, the target protein was eluted in the same number of fractions (Figure 5). Accordingly, the dynamic binding capacity at 600 cm/hr was comparable to that obtained when the column was run at 150 cm/hr (20.3 and 21.8 mg/ml, respectively). Furthermore, chromatograms could be overlapped almost perfectly, showing the rapid mass transfer characteristics of Profinity IMAC Resin. The flow properties and open pore structure of the resin favor fast mass transfer and rapid binding kinetics, which are well suited for purifying His-tagged proteins at various flow rates without compromising binding capacity or recovery.

Dynamic binding capacity results are shown in Figure 6. The amount of target protein that bound to Profinity IMAC was slightly higher than that bound to resin 1 from supplier A, and much higher than that bound to the resin from supplier B.

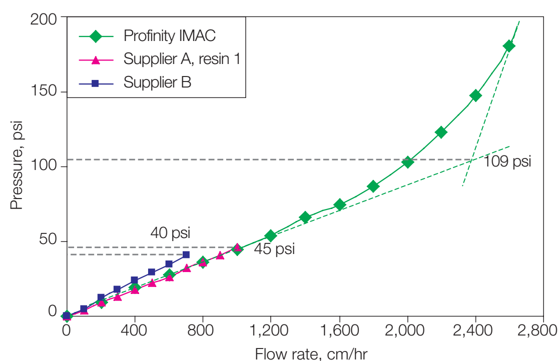


Fig. 2. Comparison of net maximum operating pressure for different IMAC resins. The pressure values shown are the estimated net maximum operating pressures for each resin. Resins were converted to a 50% (v/v) slurry in 20 mM sodium phosphate buffer and packed in a 1.1 x 30 cm Amicon Column to a bed height of 20 cm at up to 43 psi (3 bar). Flow rates were then increased stepwise by 200 cm/hr and held for 2 min at each step. The pressure-flow curve for Profinity IMAC Resin became nonlinear only at pressures above 109 psi (7.5 bar), the point defined as the intersection of the two tangents on the pressure-flow curve.

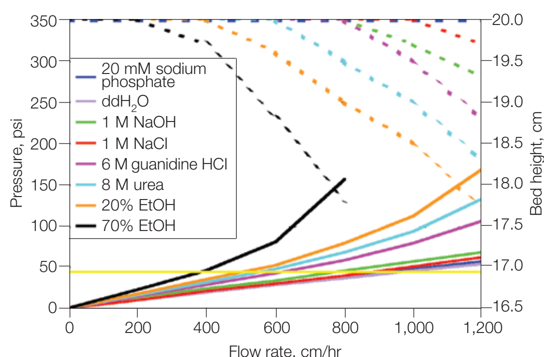
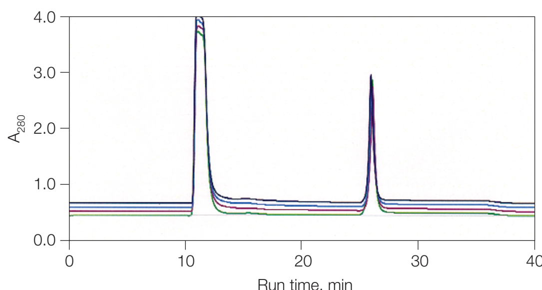


Fig. 3. Shrink/swell test on Profinity IMAC Resin. Common solutions used in His-tagged protein purification were run on a 1.1 x 30 cm Amicon Column packed with Profinity IMAC to a bed height of 20 cm. System pressure and column bed height compression were recorded for each flow rate. Flow rates were increased stepwise to 200 cm/hr and held for 2 min at each step. Yellow horizontal line indicates 43 psi (3 bar).



Stripping	50 mM EDTA, 50 mM sodium phosphate, 300 mM NaCl (pH 7.5)
Cleaning	50 mM sodium acetate, 300 mM NaCl (pH 4.0)
Charging	100 mM $NiSO_4$ (pH 4.0)
Cleaning	50 mM sodium acetate, 300 mM NaCl (pH 4.0)
Equilibration	50 mM sodium phosphate, 300 mM NaCl (pH 8.0)
Sanitization	1.0 N NaOH (step skipped before sample injection)

Fig. 4. BioLogic DuoFlow overlay report of a cycling study. A 1 ml column packed with Profinity IMAC was subjected to 201 cycles of use. Sample (250 µl lysate containing 3.51 mg of a 75 kD His-tagged protein) was loaded onto the column at cycle 1 and after every interval of 50 wash cycles, which included the steps described.

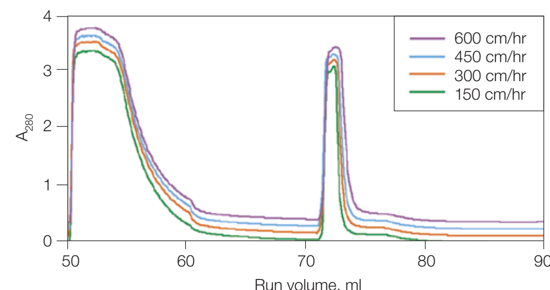


Fig. 5. Consistent binding capacity and selectivity of Profinity IMAC at different flow rates. Crude *E. coli* lysate containing an expressed 75 kD His-tagged protein was loaded onto a 1 ml column preequilibrated with loading buffer (50 mM potassium phosphate and 0.3 M NaCl, pH 8.0). The protein was eluted with the same buffer + 500 mM imidazole. Elution was monitored at 280 nm.

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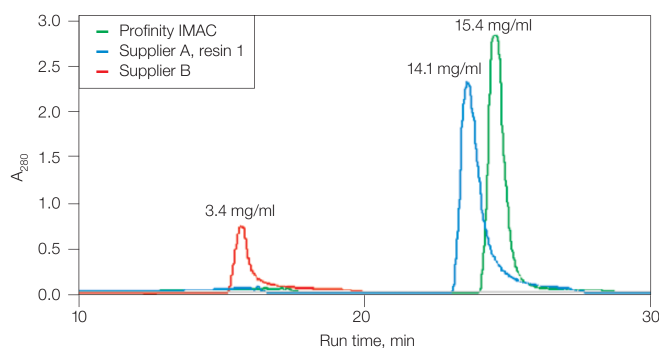
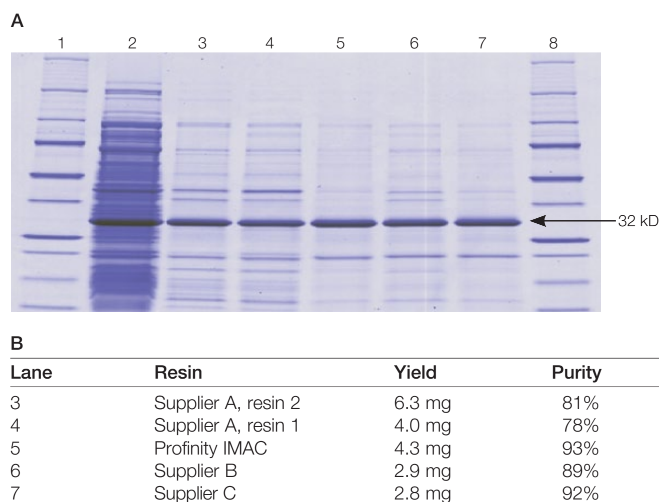


Fig. 6. Dynamic binding capacity of different IMAC resins under nondenaturing conditions. The same sample was applied until 10% breakthrough occurred. Each column was washed, then the His-tagged protein eluted to calculate the amount bound (indicated above respective peaks; the chromatograms of the elution profiles have been overlaid).



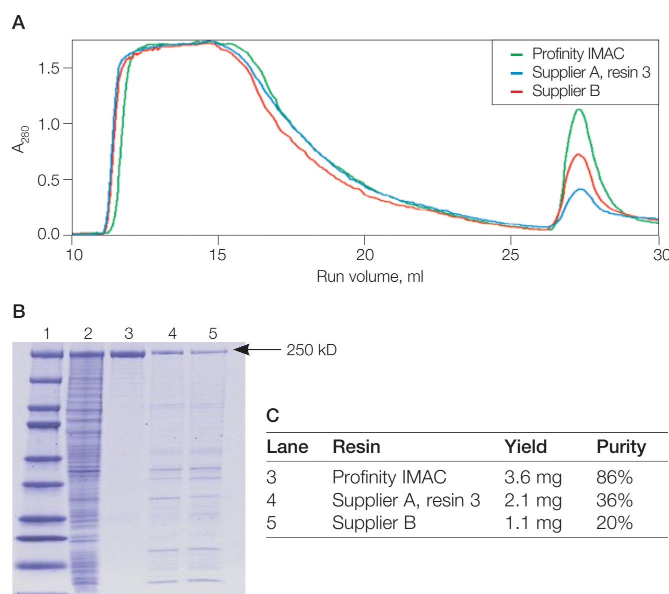
B

Lane	Resin	Yield	Purity
3	Supplier A, resin 2	6.3 mg	81%
4	Supplier A, resin 1	4.0 mg	78%
5	Profinity IMAC	4.3 mg	93%
6	Supplier B	2.9 mg	89%
7	Supplier C	2.8 mg	92%

Fig. 7. Purification of an insoluble 32 kD His-tagged protein under denaturing conditions using different Ni-charged IMAC resins. Lysate from *E. coli* expressing a His-tagged *Anabaena* protein was loaded onto Micro Bio-Spin Columns containing IMAC resins equilibrated with loading buffer (50 mM sodium phosphate, 0.3 M NaCl, 8 M urea, pH 8.0). His-tagged proteins were eluted in the same buffer + 250 mM imidazole. **A**, 3 µg of crude lysate or eluted protein separated by SDS-PAGE. Lanes 1 and 8, 10 µl Precision Plus Protein™ Standards; lane 2, crude lysate; lane 3, resin 2 from supplier A; lane 4, resin 1 from supplier A; lane 5, Profinity IMAC Resin; lane 6, resin from supplier B; lane 7, resin from supplier C). **B**, calculated yield and purity of eluted His-tagged protein.

Yield and Purity of His-Tagged Proteins

High target protein purity, the most important attribute of a particular resin, results from a high stringency of interaction at the resin's optimal ligand density. Profinity IMAC specifically selects for recombinant His-tagged proteins over naturally occurring His-containing proteins; its high selectivity resulted in greater target protein purity than obtained with other IMAC resins (Figure 7). Profinity IMAC's open pore structure especially favors purification of large His-tagged proteins, as seen by a comparison of the dynamic binding capacities of Profinity IMAC and other IMAC resins for a 250 kD protein (Figure 8A). Target protein purity of up to 86% was obtained using Profinity IMAC Resin (Figures 8B and 8C).



C

Lane	Resin	Yield	Purity
3	Profinity IMAC	3.6 mg	86%
4	Supplier A, resin 3	2.1 mg	36%
5	Supplier B	1.1 mg	20%

Fig. 8. Purification of a 250 kD His-tagged protein on different IMAC resins. A, overlaid chromatograms. A lysate of *E. coli* expressing the protein was loaded at 0.2 ml/min onto Bio-Scale Columns containing 1 ml resin equilibrated with 5 CV of buffer (50 mM KH_2PO_4, 0.3 M KCl, 10% glycerol, 6 M urea, pH 8.0) + 5 mM imidazole. The columns were reequilibrated (6 CV), washed with 7 CV of buffer + 10 mM imidazole, and eluted at 1 ml/min with 6 CV of buffer + 250 mM imidazole. **B**, SDS-PAGE of the pooled, desalted fractions containing the eluted His-tagged protein. Lane 1, Precision Plus Protein Standards; lane 2, crude extract; lanes 3–5, eluates from Profinity IMAC, resin 3 from supplier A, and resin from supplier B. **C**, calculated yield and purity of the eluted His-tagged protein.

Conclusions

When charged with Ni^{2+} ions, Profinity IMAC Resin offers highly selective binding of His-tagged proteins. Columns packed with Profinity IMAC Resin sustained high pressure from increased buffer viscosity due to denaturing agents, and exhibited excellent reproducibility over 200 cycles of use. Improved productivity for His-tagged protein can be achieved using Profinity IMAC, since operation at linear velocities up to 600 cm/hr did not compromise resolution. This affinity resin is compatible with common reagents used in protein purification, and is stable across a very broad pH range. Profinity IMAC is appropriate for the separation of His-tagged proteins from various expression systems under native or denaturing conditions, using liquid chromatographic instrumentation, gravity-flow columns, or spin columns. Profinity IMAC Resin offers superior performance over several other commercially available IMAC resins.

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Bulletin 5456 Rev B US/EG

15-0498 0415 Sig 1214