Application Forum

Roche's New One-Step cComplete His-Tag Purification Resin

Introduction

Roche's innovative **cComplete His-Tag Purification Resin** is a high-capacity immobilized metal ion affinity chromatography (IMAC) matrix for convenient single-step purification of His-tagged proteins from lysates.

Proprietary nickel-chelate chemistry is compatible with reducing agents, such as DTT, chelating metalloprotease inhibitors, such as EDTA, and many different buffers and salt conditions. This wide range of compatible ingredients makes it easy to optimize buffers for high protein stability and solubility, protecting proteins from proteases and oxidation.

In contrast to currently available nitrilotriacetic acid (NTA) resins or resins using chelators such as iminodiacetic acid (IDA), nickel ions are immobilized on cComplete His-Tag Purification Resin using one of the strongest chelators known. This new chemistry minimizes nickel ion leakage, even with EDTA and DTT containing buffers. In addition to eliminating the need for resin recharging, the resin stabilizes target proteins by preventing nickel ions from catalyzing protein oxidation.

Materials and Methods

Experiment #1: cComplete His-Tag Purification Resin and two commercially available resins using Ni-NTA (Supplier Q) and another chelator (Supplier G) were incubated for one hour at room temperature (+15 to +25°C) in nine volumes of buffer containing 10 mM EDTA, 10 mM DTT, 500 mM imidazole, 300 mM NaCl, and 50 mM NaH_2PO_4 , at pH 8.0. The amount of nickel ions released was measured by ICP-MS.

Experiment #2: Two milliliters of native lysate containing moderate amounts of His₆-MBP or His₁₀-T4 DNA Ligase were incubated for two hours with 50 or 40 µl of cComplete His-Tag Purification Resin in a buffer (150 mM NaCl, 50 mM Tris-HCl pH 7.5, 2 mM DTT) containing 5 or 10 mM imidazole. Unbound material was eluted in buffer A supplemented with 400 mM imidazole.

Results

Experiment #1: As shown in Figure 1, cComplete His-Tag Purification Resin lost less than 1% of nickel ions. In comparison, resins from Suppliers Q and G released 76% and 59% of their nickel ions.

Experiment #2: As shown in Figure 2, cComplete His-Tag Purification Resin purified proteins after just one run. This sepharose-

Figure 1: Loss of resin Ni ions under stringent conditions by different supplier resins.

Figure 2: Efficiently purify His₆- (panel A) and His₁₀-tagged (panel B) proteins.

based, pre-charged and ready-to-use Ni²⁺-chelate matrix purifies proteins from crude lysates using a simple one-step process.

Discussion

Users will appreciate the buffer flexibility for high protein stability and function. The resin's high binding capacity and specificity are ideal for one-step purifications. cComplete His-Tag Purification Resin is designed for the production of large amounts of highly purified proteins. It shows negligible nickel leakage which reduces toxic nickel waste, requiring less nickel recharging, a costly and time-consuming process.

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Sponsored Paper. *BioTechniques* 53:63 (July 2012)
doi 10.2144/000113893