Purification of Cas9 at ~99% Purity in a Single Chromatographic Step

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Abstract:

The **Cas9** enzyme (MW ~144 kDa) is a key element of CRISPR (Clustered regularly interspaced short palindromic repeats) gene editing technology, and its purity is directly correlated to CRISPR editing efficiency. It has been shown that compared to viral methods, direct delivery of assembled ribonucleoproteins (RNPs) is preferable for genome editing in cells and animals because of reduced off-target effects, lower toxicity, and higher editing efficiency. Cas9 purity is especially important for trials that test the ability to edit genes in living human cells *in vivo,* and for potential clinical applications. Previously, extra-pure samples could only be obtained using a method with 4-5 chromatographic steps. Recently, researchers at Hubei University (Wuhan, China) used a novel protein tag technology to streamline the production of Cas9 ribonucleoproteins in one step, saving 2-3 days and increasing yields fourfold over existing methodology. The purification system is based on the ultra-high-affinity interaction between a variant of the Colicin E7 DNAse (CL7) and its inhibitor, Immunity protein 7 (Im7) ($K_D = 10^{-14} - 10^{-17}$ M). The Im7 inhibitor is covalently crosslinked to agarose beads, while the CL7 domain is expressed as a fusion tag with the target (Cas9) protein. They were able to purify Cas9 RNPs to 89% purity in a single chromatographic step. Here we report using the CL7/Im7 purification system with optimized reagents and methods to purify extra-pure (99%) samples of Cas9 in one chromatographic step.