

Development of Small-Scale, High-Throughput, Chromatographic Protein Purification

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Abstract:

High-throughput purification methods are needed to support fermentation development and process validation exercises in order to increase the capacity of process development groups. In this example, a representative lab-scale purification of an intracellularly expressed recombinant protein required three days and a minimum of 10 g wet cell weight (wcw). To reduce these resource demands, a high-throughput purification process was developed which requires an input of only 0.3 g (wcw). This process utilizes mechanical lysis with silica beads and clarification by centrifugation. The clarified lysate is further purified by two automated chromatography steps on a Tecan Freedom EvoR workstation using PhyTip[®] disposable pipet tips pre-filled with microliter volumes of resin. The high-throughput process is consistent, with variability of less than 20% between duplicate purifications with respect to protein purity and yield. In addition, the yield and purity correlate with lab-scale runs for varying fermentation conditions, validating its utility. Using this high-throughput purification process approximately 100-fold more conditions can be evaluated compared to the lab-scale process or alternatively, an equivalent number of conditions can be evaluated in 1% of the time.