

Analysis of selective metal-salt-induced endotoxin precipitation in plasmid DNA purification using improved LAL assay and central composite design

Abstract

Recent advancements in plasmid DNA (pDNA) production involve the development of innovative and cost-effective methods as well as reduced number of unit operations. This study investigates the feasibilities of using a metal salt to selectively remove endotoxins from clarified cell lysates containing plasmid DNA. Screening of endotoxin precipitation in various metal salt solutions and optimization of process conditions (pH, ion concentration, temperature, and incubation time) using central composite design experiments have been carried out successfully. Results show that selective endotoxin precipitation (<0.05 EU/ μ g) can economically be carried out during the alkaline cell lysis procedure (neutralization step) at a pH condition similar to that of alkaline-lysed cell lysate, a low ZnSO₄ concentration (0.5 M), a minimum incubation time (30 min), and a temperature of 15 °C. In summary, this method provides ease of subsequent plasmid DNA purification due to reduced bulk impurities and cost-effective and most importantly high endotoxin removal ($>80\%$) and plasmid recovery ($>90\%$).