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Process Development of pVAX1/lacZ Plasmid DNA Purification Using Design of Experiment

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Abstract : Third generation of vaccines is based on gene therapy where DNA is introduced into patients. The antigenic or therapeutic proteins encoded from transgenes DNA triggers an immune-response to counteract various diseases. Moreover, DNA vaccine offers the customization of its ability on protection and treatment with high stability. The production of DNA vaccines become of interest. According to USFDA guidance for industry, the recommended limits for impurities from host cell are lower than 1%, and the active conformation homogeneity supercoiled DNA, is more than 80%. Thus, the purification strategy using two-steps chromatography has been established and verified for its robustness. Herein, pVax1/lacZ, a pre-approved USFDA DNA vaccine backbone, was used and transformed into E. coli strain DH5α. Three purification process parameters including sample-loading flow rate, the salt concentration in washing and eluting buffer, were studied and the experiment was designed using response surface method with central composite face-centered (CCF) as a model. The designed range of selected parameters was 10% variation from the optimized set point as a safety factor. The purity in the percentage of supercoiled conformation obtained from each chromatography step, AIEX and HIC, were analyzed by HPLC. The response data were used to establish regression model and statistically analyzed followed by Monte Carlo simulation using SAS JMP. The results on the purity of the product obtained from AIEX and HIC are between 89.4 to 92.5% and 88.3 to 100.0%, respectively. Monte Carlo simulation showed that the pVAX1/lacZ purification process is robust with confidence intervals of 0.90 in range of 90.18-91.00% and 95.88-100.00%, for AIEX and HIC respectively.

Keywords : AIEX, DNA vaccine, HIC, puification, response surface method, robustness
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