Optimization of the Human p50/Rel A Protein Protocol for Investigation of the Role of Its Intrinsically Disordered Domain

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TBIOMD 499

Proteins in the NFκB family are transcription factors that modulate the expression of genes relating to immunity and inflammation. One protein within this family is the p50/RelA heterodimer which includes a structured DNA-binding domain and a Transcription Activation Domain (TAD) which is intrinsically disordered, or naturally lacking secondary and tertiary structure. While it was historically thought that the DNA-binding domains and TADs of NFκB proteins behave independently of each other, previous studies have shown that the presence of a TAD may impact DNA binding affinity and specificity in a shortened p50/RelA protein. With the goal of further assessing and comparing the roles of the intrinsically disordered domains in the proteins of this family, we have worked to troubleshoot and optimize protocols relating to expressing and purifying a full-length, human construct of p50/RelA. We assessed multiple chromatography steps and introduced changes to improve yield. Further optimization is needed to improve the expression and cation exchange chromatography steps in order to obtain intact, full-length protein. Optimization of these protocols will enable investigations of the role of the TAD, including its effects on DNA binding and role of coactivator interactions, in a construct of p50/RelA that resembles that within the human body.