p50 and RelA are crucial NF-kB transcription factor family subunits, forming heterodimers that manage cell survival, proliferation, function, and inflammation. While past biochemical research has been conducted using mouse p50/RelA dimers, their relevance to human health remains to be determined. This prompts the scientific question of how the biochemical functions of the mouse proteins compare to the human proteins. We used protocols to express and purify human and mouse p50/RelA dimers for structural and functional analysis. In the first stage, recombinant protein expression and affinity chromatography were used for purification, followed by SDS-PAGE to assess molecular weight and stability. We successfully expressed human and mouse p50/RelA proteins in E. coli and purified them using Ni2+ affinity chromatography, showing positive results with dark bands on SDS-PAGE gels. However, impurities on the gels indicate some unwanted residual proteins remain, suggesting incomplete isolation of Rel A and p50. To improve the removal of unwanted proteins, we modified the wash step of Ni2+ affinity chromatography by increasing imidazole concentration in the wash buffer. These changes are important because higher imidazole concentrations can help elute non-specifically bound contaminants without affecting the target proteins. However, we found this change to the protocol did not improve our yield or purity. Further improving the experimental protocol to achieve better separation will enable comparison of NF-kB pathway functionality between human and mouse RelA and p50 proteins by making it easier to apply findings from mouse models to human health, providing better insights and potential therapeutic approaches.