

Recombinant chicken IL-8 orthologue, CXCLi1

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Interleukin-8 (IL-8) is a chemokine that facilitates trafficking of neutrophils when the host is challenged by pathogens such as *Salmonella enterica*. Two chicken IL-8 orthologues exist, CXCLi1 and CXCLi2, but there are no commercial antibodies for these proteins. CXCLi1 and CXCLi2 share 48% and 50% amino acid identity with human IL-8. Previous research shows that polyclonal antibody against human rIL-8 will cross-react with protein from *Salmonella* infected chicken cells. Unfortunately, we are unable to determine which, if either, chicken orthologue is reacting to these antibodies. Furthermore, without more specific reagents we cannot identify functional activities, differences or redundancies between the two chicken orthologues. Consequently, for this project we generated a recombinant CXCLi1 (rCXCLi1) vector and purified protein. Reverse transcriptase was used to generate cDNA obtained from a chicken cell line that was infected with *Salmonella enterica* serovar Typhimurium. PCR was then used to retrieve the CXCLi1 specific cDNA and this material was ligated into a pET30a expression vector, transformed into *E. coli*, and sequenced for confirmation. Protein expression induced with IPTG and SDS-PAGE and western blot techniques (anti-His tag) confirmed the presence of a protein of the predicted mass (~12 kD). We will determine if antibody against human rIL-8 recognizes our rCXCLi1 and proceed with development of anti-rCXCLi1 antibody that will be used for development of an ELISA assay and transwell migration neutralization assay. We will also begin work on a rCXCLi2 vector for future functional analyses and comparison with rCXCLi1.