

# Recombinant Synthesis of Hyaluronidase With Modified IgE-Binding Epitope for the Purpose of Reduced Allergenicity

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Immunotherapy involves exposing patients to small amounts of the allergen to desensitize their immune systems. This can be dangerous to patients who experience anaphylactic reactions to the allergen. The advent of recombinant technology creates the possibility of engineering biosimilar proteins that are less allergenic than the original and, thus, safer to use in immunotherapy. This study aims to modify an immunoglobulin E (IgE) binding site of hyaluronidase (Apm 2) protein in bee venom to reduce the ability of the IgE antibody to bind to this site. We used Genscript to develop four plasmids encoding biosimilar variants of hyaluronidase, modified at the IgE binding site, with various degrees of mutations from conservative replacement of amino acids to deletion of the binding site. Mutations were selected based on simulations run on AlphaFold, which helped identify the structural impact of the mutations. We expressed the native protein and these modified variants of hyaluronidase by transforming *Escherichia coli* (*E. coli*) with our plasmids. We evaluated their allergenicity using an enzyme-linked immunosorbent assay (ELISA). We extracted the protein and measured the yield via a competitive ELISA that detects polyhistidine tags (his-tag). Then, we ran an indirect ELISA using a chimeric monoclonal IgE antibody that binds to the specific binding site we modified. Results indicate that the more severe the mutation, the less affinity the antibody has for the protein's binding site. The next steps would include testing the variants on human sera. We hope this project will further knowledge of potential immunotherapy options for anaphylactic reactions.