

Contract work case study

Expression and purification of an intracellular protein identified as a gene therapy target,

Remit:

Develop a custom exclusive purification protocol capable of production of 50mg of a pure human intracellular protein for use in immuno and activity assays for a clinical trial. The project was managed by Dr Holger Schuhmann, The Native Antigen Company's lead purification expert

Stage 1 - DNA synthesis, cloning, plasmid and protein yield and expression studies (9 weeks)

24 permutations of optimised DNA sequences, bacterial host cell lines and vector backbones were assessed for plasmid yield in mini, midi and giga prep sizes.

Cryptic bacterial promoters that had a deleterious effect on plasmid yield were identified for many constructs, enabling selection of a lead plasmid candidate in a permissive bacterial strain that showed good yield at gigaprep scale, with a 5x increase in protein expression over initial constructs.

SDS-PAGE and Western blots were presented to enable a stop/go decision prior to scale up by the customer, thereby minimising upfront costs and risk.

Stage 2: Scaleup and Purification research

The lead candidate plasmid was prepared in bulk and large-scale transient transfection of human suspension cells was performed to supply bulk raw material for purification research.

Lysis methods were investigated and optimised, then a purification method was developed. ELISA and Western blot demonstrated that the histidine tag was occluded in the lead candidate construct; accessible when denatured but not when native during chromatography. Rapid recloning was performed to add a linker sequence into the DNA construct, and bulk preparations were repeated.

Binding of the protein was then successful and a purification method resulting in 95%+ pure protein was developed. Activity testing was performed using an outsourced customer assay, all other QC assays were performed in house. Using the resultant yield calculations a price per mg for future production was agreed to enable production of 50mg for the clinical trial.

Figure below: Final purified protein



"Thanks for all the hard work you put in on this difficult protein. We will definitely use you again on our next project"

- Case study customer