

protein using AlphaFold2.

A PoAstV4 spike construct containing residues 420 to 655 was designed rom the full-length capsid protein of

PoAstV4. The recombinant PoAstV4 spike protein expressed in *E. coli* resulted in high yields of soluble protein

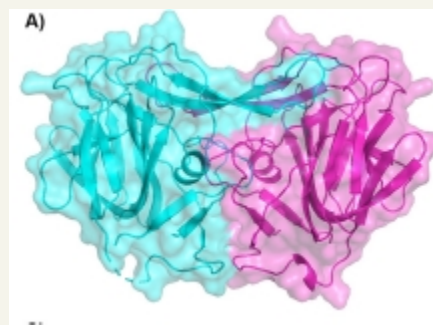
(>12 mg / Liter *E. coli*). The calculated molecular weight of the PoAstV4 spike is 27.8 kD (+1.6 kD cleavable

histidine affinity tag). This was confirmed using SDS-PAGE analysis of purified PoAstV4 spike. Size

exclusion chromatography showed purified PoAstV4 spike protein eluted at the approximate size of a homodimer,

which suggests that the recombinant protein is folded correctly.

The PoAstV4 capsid spike crystal structure reveals a homodimeric protein formed of mainly beta-strands, similar to other animal and human astrovirus capsid spikes, and consistent with in-solution studies with size-exclusion chromatography (FIG. 2B). The structure confirms that the predicted PoAstV4 spike residues 420 to 655 form the structural domain. Interface analysis using the PDBePISA server reveals 4660 Å buried at the dimer interface, with 64-65 interacting residues in each chain.

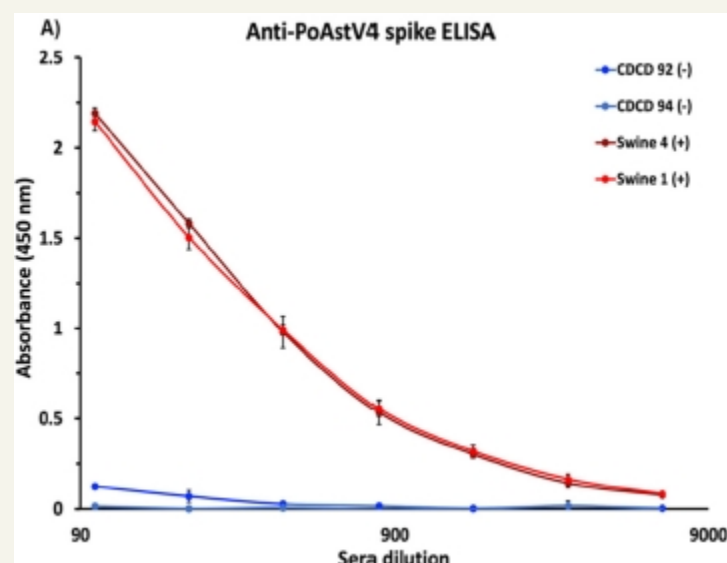


An enzyme-linked immunosorbent assay (ELISA) was developed to determinine if the recombinant PoAstV4 spike

could be used to detect PoAstV antibodies. Serum samples collected during an outbreak of PoAstV4 in Iowa showed

IgG reactivity towards the PoAstV4 spike antigen. Sera from presumed seronegative cesarean-derived, colostrum-

deprived (CDCD) pigs, which do not receive maternal antibodies, exhibited minimal reactivity.



APPLICATIONS

- Serum assays for porcine astrovirus infection.
- ELISA
- Positive control
- Immunogenic agents for porcine astrovirus spike proteins
- For raising antibody reagents
- Polyclonal antisera

- ▶ Monoclonal antibodies
- ▶ For immunoprotection
- ▶ Vaccine development

ADVANTAGES

- ▶ Specific to the porcine astrovirus 4 genotype: a very common genotype of porcine astrovirus
- ▶ PoAstV4 causes both GI and respiratory symptoms

INTELLECTUAL PROPERTY INFORMATION

Patent Pending

RELATED MATERIALS

ADDITIONAL TECHNOLOGIES BY THESE INVENTORS

- ▶ [Glycoengineering Of The Foldon Protein Trimerization Domain To Shield It From Antibody Immune Responses](#)
- ▶ [Human Astrovirus Neutralizing Monoclonal Antibody Sequences](#)
- ▶ [Simplified Workflow For Hybridoma Antibody Sequencing](#)
- ▶ [Methods To Rapidly Measure Antibodies And Other Biomolecules In Clinical Specimens Utilizing Biolayer Interferometry](#)

University of California, Santa Cruz
Industry Alliances & Technology Commercialization
Kerr 413 / IATC,
Santa Cruz,CA 95064

Tel: 831.459.5415
innovation@ucsc.edu
<https://officeofresearch.ucsc.edu/>
Fax: 831.459.1658

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