

## Purification and Characterization of Nipah Virus Nucleocapsid Protein Produced in Insect Cells

### ABSTRACT

The nucleocapsid (N)protein of Nipah virus (NiV) is a major constituent of the viral proteins which play a role in encapsidation, regulating the transcription and replication of the viral genome. To investigate the use of a fusion system to aid the purification of the recombinant N protein for structural studies and potential use as a diagnostic reagent, the NiV n gene was cloned into the pFastBacHT vector and a his tagged fusion protein was expressed in Sf9 insect cells by recombinant baculovirus. Western blot analysis of the recombinant fusion protein with anti-Niv antibodies produced a band of approximately 62 kDa. A time course study showed that the highest level of expression was achieved after 3 days of incubation. Electron microscopic analysis of the NiV recombinant N fusion protein purified on a nickel-nitrilotriacetic acid resin column revealed different types of structures, including spherical, ring-like, particles. The light-scattering measurements of the recombinant N protein also confirmed the polydispersity of the sample with hydrodynamic radii of small and large types. The optical density spectra of the purified recombinant fusion protein revealed a high A<sub>260</sub> / A<sub>280</sub> ratio, indicating the presence of nucleic acids. Western blotting and enzyme-linked immunosorbent assay results showed that the recombinant N protein exhibited the antigenic sites and conformation necessary for specific antigen-antibody recognition.