

Contract work case study – in vitro maturation of Zika VLPs

Zika virus (ZIKV) is a mosquito borne flavivirus. Originally discovered in the Zika forest in Uganda, ZIKV has caused multiple smaller outbreaks in Micronesia and Polynesia. In 2015 ZIKV was introduced into South America, causing an epidemic that prompted the WHO to declare a health emergency in 2016.

To provide key reagents for the development of diagnostic assays and vaccines The Native Antigen Company has produced recombinant Zika virus-like particles (ZIKV-VLPs). ZIKV-VLPs contain the two flaviviral envelope proteins Envelope (E) and pre-Membrane (prM). During natural infection, immature virus particles pass through the Golgi network where they are activated by low pH and the Golgi resident endoprotease furin, which cleaves prM into Membrane protein (M), which is retained in the virion, and the pre-peptide. Due to a re-arrangement of the virion envelope following furin cleavage and a pH shift from low pH in the Golgi network to neutral pH, virions are very prone to aggregation.

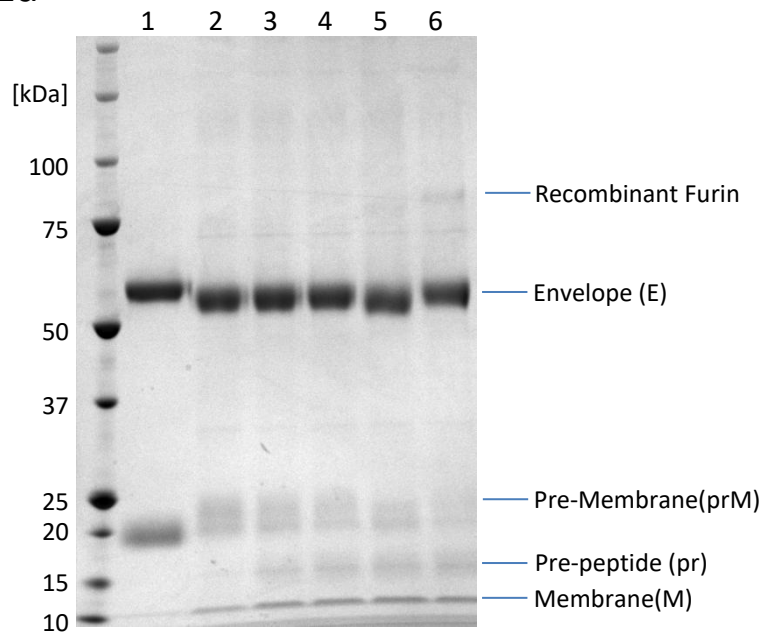
Human embryonic kidney 293 (HEK293) cells are known to produce very low levels of endogenous furin which allows the production of immature ZIKV-VLPs that do not aggregate even at high concentrations. Therefore, NAC has decided to produce ZIKV-VLPs recombinantly in HEK293 cells.

NAC was approached by company X to provide mature ZIKV-VLPs for electron microscopy analysis.

NAC decided to produce mature ZIKV-VLPs by *in vitro* maturation of immature ZIKV-VLPs using treatment with furin. To demonstrate the feasibility of this approach ZIKV-VLPs were incubated with increasing amounts of recombinant furin in the presence of calcium at pH5.0. The resulting particles were analysed by SDS-PAGE (Fig. 1a). As a control non-treated Dengue 2 virus like particles (DENV2-VLP) were used. With increasing amounts of furin increasing amounts of prM were cleaved into M and pre-peptide. Densitometry analysis (Fig. 1b) shows that maturity levels could be increased from ~35% to ~86% using in vitro maturation.

To fulfil the customers requirement for 100ug of mature ZIKV-VLP, 150ug of ZIKV-VLP were treated with furin. The reaction was spun, and pellet and supernatant were then analysed on SDS-PAGE (Fig. 2).

Fig. 1a



- 1: DENV2-VLP – untreated
- 2: ZIKV-VLP – untreated
- 3: ZIKV-VLP – 1U furin
- 4: ZIKV-VLP – 2U furin
- 5: ZIKV-VLP – 4U furin
- 6: ZIKV-VLP – 8U furin

Fig. 1b

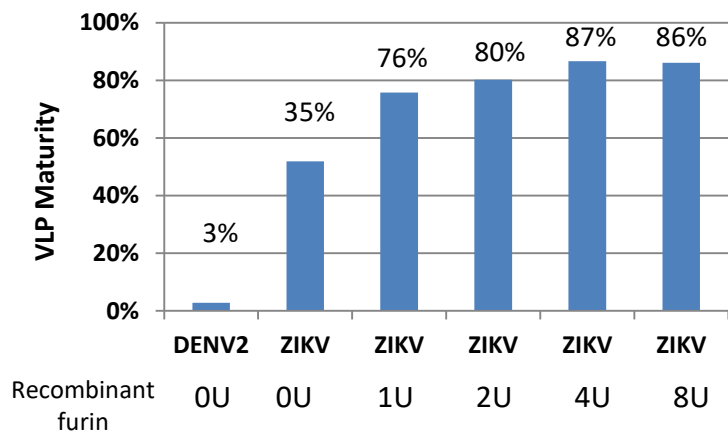


Fig. 2

