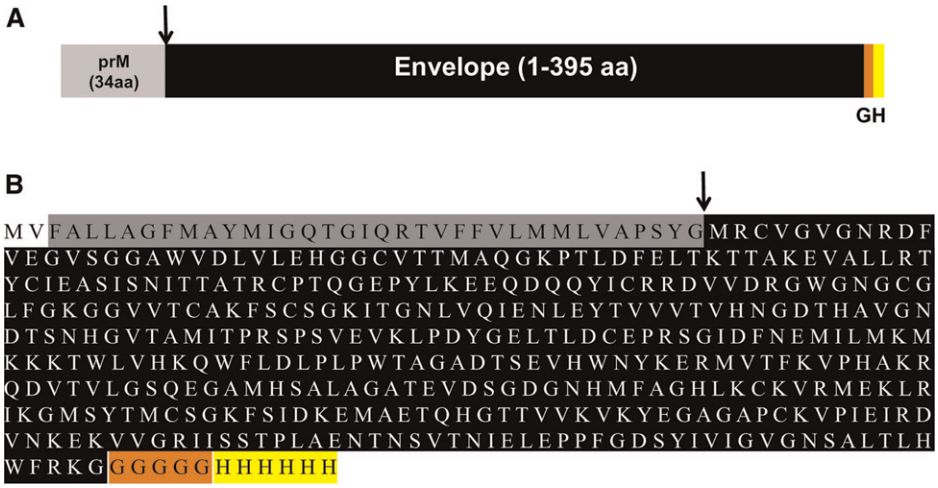
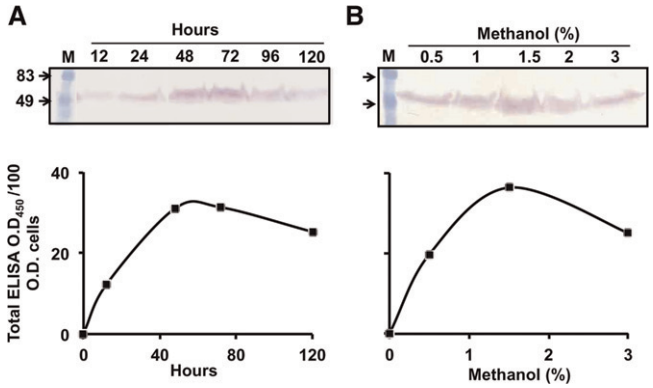


Supplemental Figure 1. Design of DENV-4 E antigen. (**A**) Representation of the *DENV-4 E* gene design and (**B**) the aa sequence encoded by it. The 34 aa of prM, 395 aa of E of DENV-4, pentaglycl linker, and hexa-histidine tag (in order from N-terminus to C-terminus) are represented in grey, black, orange, and yellow, respectively. The first two aa residues (MV) were introduced to place the translational initiator codon in the context of Kozak consensus sequence. The downward arrows in (**A**) and (**B**) denote the prM signal cleavage site determined by N-terminal sequencing of the purified DENV-4 E. aa = amino acid; DENV-4 E = dengue virus serotype 4 ectodomain.



Supplemental Figure 2. Optimization of methanol percentage and hours of induction for optimal protein expression. (**A**) *DENV-4 E* gene-harboring *Pichia pastoris* clone was grown to logarithmic phase and induced with 1% methanol. Subsequently, aliquots were taken at 12 (lane 2), 24 (lane 3), 48 (lane 4), 72 (lane 5), 96 (lane 6), and 120 (lane 7) hours post-induction. These were analyzed in a Western blot (composite figure, shown at the top) and His-Sorb ELISA (best fit curve, shown at the bottom). (**B**) Several small-scale cultures of the DENV-4 E expressing *P. pastoris* clones were grown as in (**A**) and separately induced with 0.5%, 1%, 1.5%, 2%, and 3% methanol for 72 hours. Top and bottom panels represent analysis of each aliquot through Western blot and His-Sorb ELISA, respectively (the vertical axis-scale is the same for both the panels **A** and **B**). Lane M represents pre-stained protein markers, and their sizes (in kDa) are denoted on the left. DENV-4 E = dengue virus serotype 4 ectodomain; ELISA = enzyme-linked immunosorbent assay.