



## Computational Design of a Multi-Epitope Vaccine Against Nipah Virus: Bridging Immunoinformatics and Immune Protection

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**Table S1.** Comparative Analysis of Multi-Epitope Vaccine Constructs for Nipah Virus.

Study	Target Protein(s)	No. of CTL Epitopes	No. of HTML Epitopes	TLR Docking	Immune Simulation	Codon Optimization	Strain Coverage	Expression System
Majee <i>et al.</i> (2021) [29]	Glycoprotein G	6	8	TLR3 only	Yes	No	NiV-M	Not addressed
Mohammed <i>et al.</i> (2020) [30]	Glycoprotein G	5	7	Not done	No	No	NiV-M	Not addressed
This study	Fusion protein (NiV-B/M conserved)	8	10	TLR3 & TLR4 (ClusPro + Vina)	Yes (C-ImmSim)	Yes (J-CAT, SnapGene)	NiV-B + NiV-M	Optimized for <i>E. coli</i>

**Table S2.** Comparison of Immunoinformatics-Based Vaccine Design Studies Against Nipah Virus.

Parameter	Our Study	Kumar <i>et al.</i> [38]	Banico <i>et al.</i> [39]	Albutti [40]	Shabbir <i>et al.</i> [31]
Target Protein(s)	Fusion (F) protein	Glycoprotein (G) and nucleocapsid (N) proteins	All NiV proteins: (structural & non-structural)	V protein	Nucleoprotein (N)
Number of HTML, CTL and B cell epitopes	8, 10 and 4	Not explicitly stated	10,8 and 10	9,1 and 3	8, 11 and 5
TLR Docking	TLR3 and TLR4	TLR3, TLR7, TLR8	TLR4-MD2	Not explicitly tested	Ephrin B2 receptor
Immune Simulation	Robust humoral (IgG, IgM) and cellular (IFN- $\gamma$ , IL-2) responses, memory cells	Strong immune response, memory B/T-cell induction, high IFN- $\gamma$ /IL-2 levels	Higher antibody titers	humoral/cellular response	IgG/IgM, memory B-cells
Population Coverage	89.3%, with >92% for South Asia	Not explicitly mentioned	Not explicitly mentioned	99.74%	88.3%
Expression System	E. coli (pET-29a(+))	pcDNA™3.1/V5-His-TOPO1	E. coli (pET28(a)+)	mRNA-based	E. coli (pET-28a(+))
Adjuvant Used	50S ribosomal protein L7/L12	Cholera toxin (CT) adjuvant	Resuscitation-promoting factor E (RpfE) (TLR4 agonist)	B-defensin (TLR4 agonist) + MITD sequence (enhances MHC presentation)	50S ribosomal L7/L12
Structural Validation	94.2% residues in favored regions (Ramachandran)	90.3% residues in favored regions (Ramachandran)	90.3% favored (Ramachandran)	Not explicitly mentioned	95.9% favored

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