Newcastle disease identified as one of the notifiable avian disease by the Office International des Epizooties (OIE) caused by Newcastle disease virus (NDV). The potential of the internal viral components to function as the basis for a diagnostic system has focused interest towards the nucleoprotein of negative sense RNA viruses. The nucleoprotein of many paramyxoviruses has been reported to be highly immunogenic in nature and used to produce diagnostic ELISA. From the coding sequences of nucleoprotein gene 20 different B cell epitopes was predicted using a 3D-JIGSAW software. In the presence study, a conserved B-cell epitope of nucleoprotein with the amino acid sequence 447FLDLMRA453 was found to be located in the conserved region between 443GETQFLDLMRAVANS457 of nucleoprotein of all four vaccine viruses used in South India. The conserved region incorporating the epitope 443GETQFLDLMRAVANS457 has been synthesized as custom peptide. The three dimensional structure of the epitope identified was generated by molecular modeling using Ball View software version 1.1.1. The immunodominance of the epitope was confirmed by dot-ELISA (Qualitative) and peptide ELISA (Quantitative). Peptide ELISA developed using the entire conserved region was found to produce a consistent result with HI and Whole virus protein (WVP) ELISA. The immunodominance and immunogenicity of the epitope identified was confirmed by significant positive correlation (P<0.01) between Peptide ELISA with HI and with WVP ELISA and by also Probability Plot (PP). Based on Probability Plot, it could be appreciated that all the points cluttered towards the expected line confirming the closeness of the results. This fact is further strengthened by observation from ROC curves. The empirical ROC values for HI Vs Peptide ELISA and WVP ELISA Vs Peptide ELISA were 0.97 and 1.00 respectively which are more than the standard value 0.8 confirming the closeness of the results proving immunodominance of the epitope and utility of the epitope to serve as a marker in DIVA vaccine.