

Supplemental materials

For improving the antigenicity of HCV core antigen, random mutations of this recombinant antigen were generated and evaluated by reverse-ELISA. Several mutants with higher antigenicities were examined. They can be used to develop a highly sensitive HCV ELISA. Following supplemental material provides additional information for the strategy of antigen screening (Figure S1), the antigen's sequence identity (Figure S2), the antigen's purity (Figure S3), and the plot of double-reciprocals for kinetic constants calculation (Figure S4). More detail and important data please refer to the original texts.

Antigenicity screening by reverse-ELISA

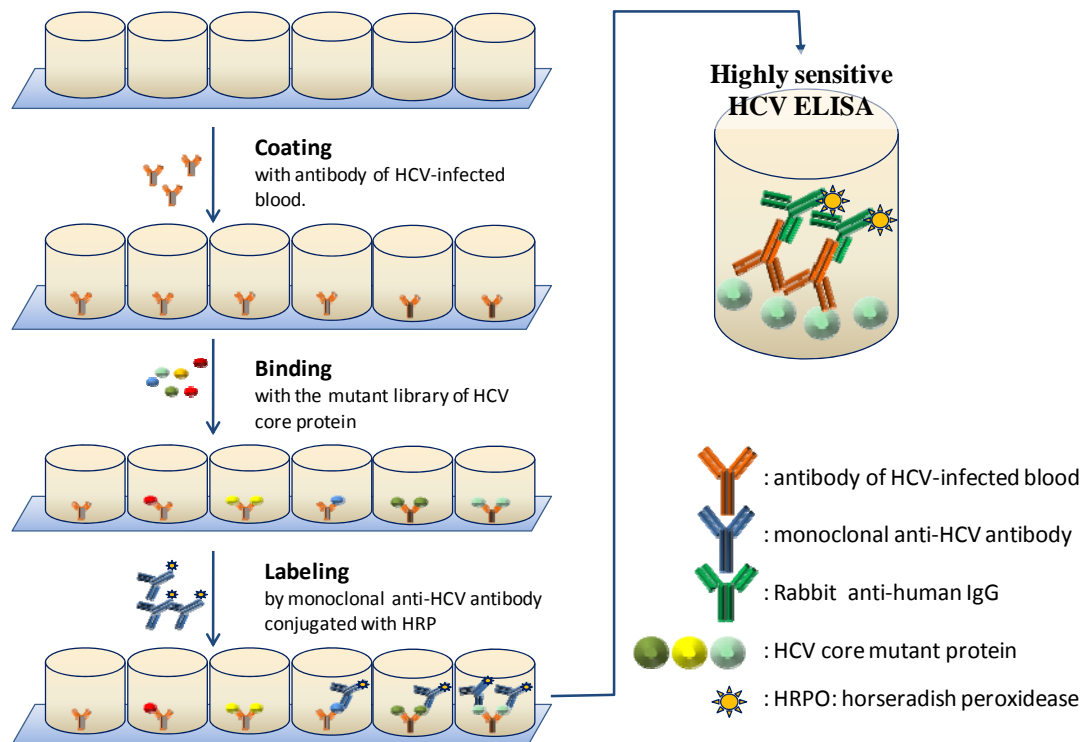


Figure S1: Schematic representation of the antigenicity screening by reverse-ELISA. After coating, binding and labeling steps, several HCV core mutants with higher antigenicities were examined for their binding capacities and affinities. These antigen are approaching for establish a highly sensitive HCV ELISA.

Figure S2: Sequence alignment of immunogen and HCV core antigens. The amino acid sequence of the chimeric HCV polyprotein as the immunogen of HCV NS3 monoclonal antibody (abcam, ab 18664-250) was aligned to six HCV core antigens using Vector NTI (Invitrogen). When compared to the immunogen sequence, It showed 91.0%, 92.6%, 91.8%, 91.8%, 91.8% and 91.8% identity for Wt, M1, M2a, M2b, M3a and M3b in order.

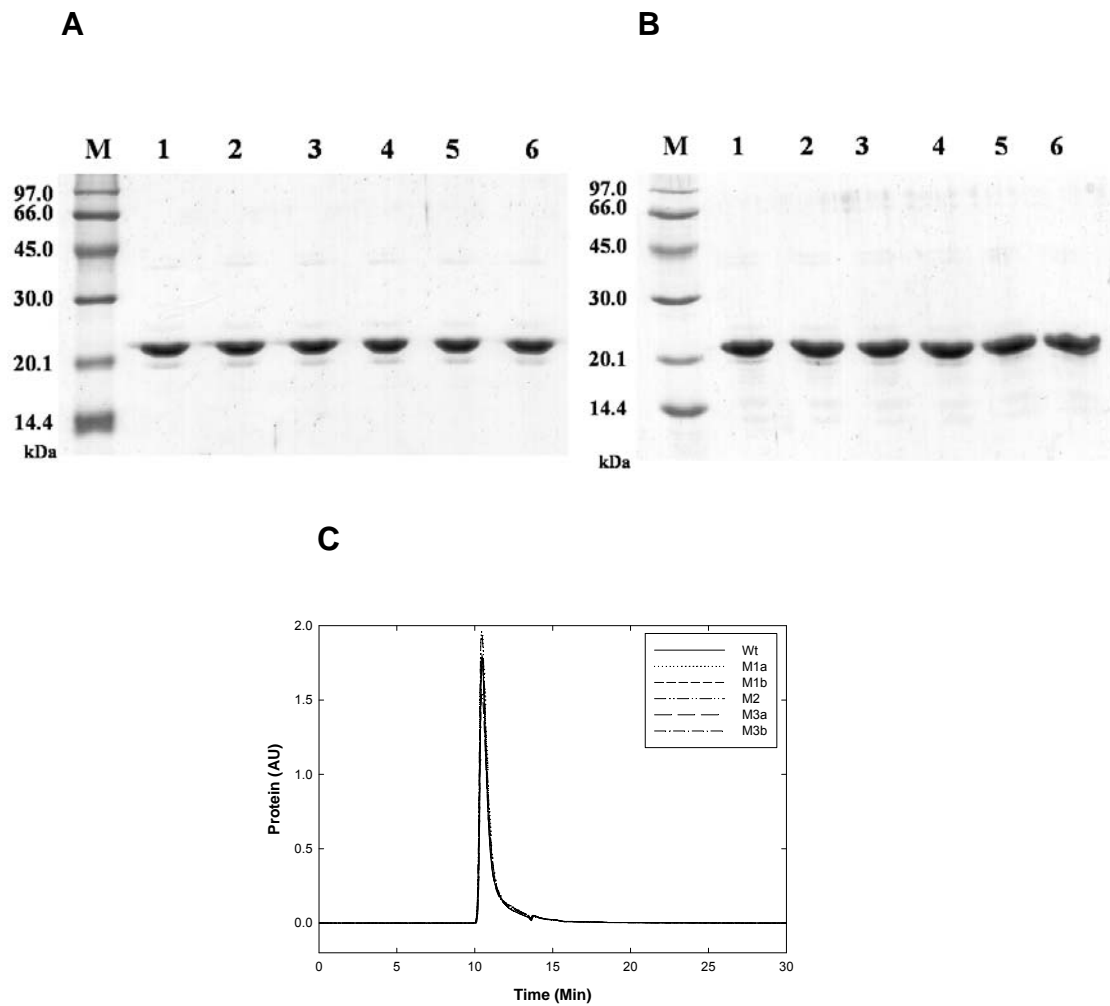


Figure S3: Purity assessment of HCV core antigens based on 15% SDS-PAGE and HPLC chromatography. Unfolded (A) and refolded HCV core antigens (B) were applied on 15% SDS-PAGE. Protein markers were run in lane M; their sizes in kDa are shown on the left. Recombinant antigens were revealed in lane 1 (Wt), lane 2 (M2), lane 3 (M3a), lane 4 (M3b), lane 5 (M1b) and lane 6 (M1a). Each antigen was applied on HPLC chromatography (C) and eluted at 10.5 minutes. The homogeneities were about 96-97% (Wt: 96%, M1a: 97%, M1b: 97%, M2: 97%, M3a: 96% and M3b: 97%).

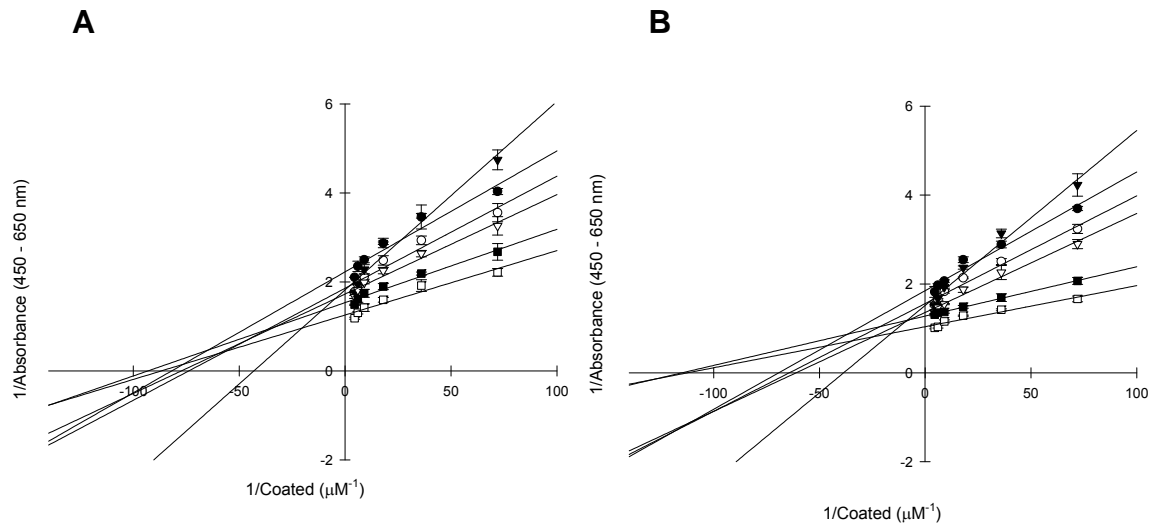


Figure S4: Plot of the reciprocals of the antigenicity versus concentration of the HCV core antigen. Data of the unfolded (A) and refolded (B) forms of HCV core antigens (Wt (●), M1a (○), M1b (▼), M2 (▽), M3a (■), and M3b (□)). These data were analyzed by modified Langmuir isotherm equation.