

digested with endoprotease Glu-c and the resulting peptides were incubated with the cross-linked antibodies to bind epitope containing peptides. The non-specific bound peptides were washed away from the beads with a neutral pH buffer while the beads were immobilized on magnet. The bound epitopes containing peptides were eluted with acidic buffer and measured on MALDI-TOF mass spectrometer. The accurate mass of mass of peptide peaks helped to identify the peptide sequence

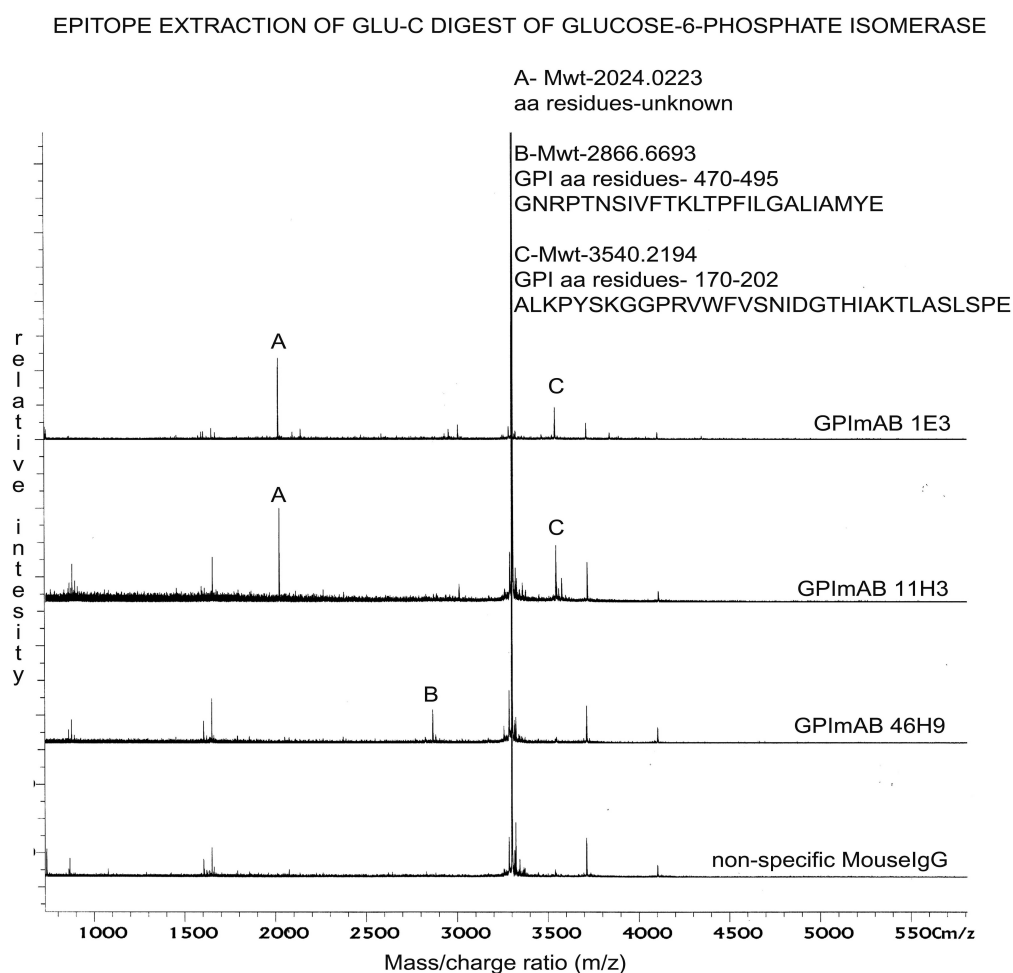


Figure 23. Mass spectrometric epitope mapping profile of epitope extracted Glu-C digested GPI.

Unique peptide peak masses were identified as epitope containing peptides. Also based on the elution profile patterns it could be once again confirmed that mAbs 11H3.C10, 1E3 recognize the same epitope and that 46H9 recognized a different epitope on GPI. The epitope peak C (MW 3540.2194) unique to 1E3 and 11H3.C10 was found to be GPI amino acid residues 170 to 202 (ALKPYSKGGPRVWFVSNIDGTHIAKTLASLSPE). The epitope peak B (MW 2866.6693) unique to 46H9 was found to be GPI amino acid residues 470 to 495 (GNRPTNSIVFTKLTPEFILGALIAMYE).

4.1.9. Protein truncation epitope mapping confirms the mass spectrometry identified epitope regions.

In order to elucidate epitopes of the GPI monoclonal antibodies truncated versions of GST-GPI - i. GPI-I (GPI amino acid residues 1-190); ii. GPI-II (GPI amino acid residues 1-368); iii. GPI-III (GPI amino acid residues 324-559); IV. GPI-IV (GPI amino acid residues 485-559) were generated. The expressed proteins were used in western blot analysis with the GPI mAbs. It could be shown that 11H3.C10 and IE3 reacted to GST-GPI (1-368) only and do not react with GST-GPI(1-190) meaning that these antibodies have epitopes lying within GPI amino acid residues 170-324. The 46H9 antibody reacted only to GST-GPI(324-559) and showed no reactivity to GST-GPI(485-559) showing that its epitopes lies between GPI residues 324-485. Also to be noted is that the epitope residues identified by mass spectrometric epitope mapping for 1E3/11H3.C10 (170-202) and for 46H9 (470-495) are also exclusively present only in the truncated GPI clones reacting with antibodies on western blots.

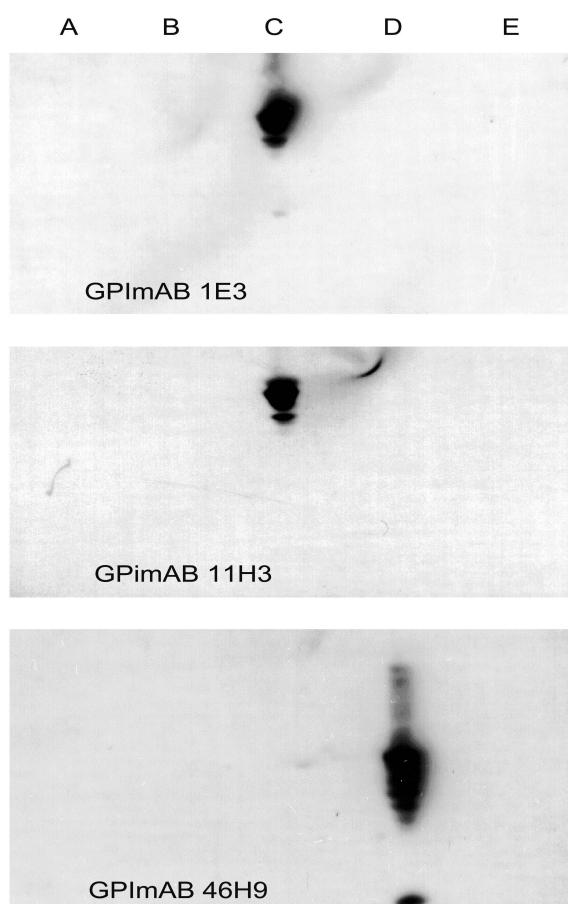


Figure 24. Western blot showing the reactivities of mGPI mAb to expressed truncated proteins of mouse GPI. The lanes were loaded with bacterial lysates expressing (A) GST. (B) GST-mGPI(1-190). (C) GST-mGPI(1-368). (D) GST-mGPI(324-559). (E) GST-mGPI(489-559).