

**PRODUCTION OF THE FIRST HUMAN MONOCLONAL ANTIBODY  
HIGHLY SPECIFIC FOR HPA-5B ALLOANTIGEN (F2H11).  
APPLICATION FOR THE PHENOTYPING OF PLATELETS**

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Neonatal AlloImmune Thrombocytopenia (NAIT) is the commonest cause of severe isolated thrombocytopenia in the foetus and newborn, occurring in about 1/800 live births. NAIT can occur before 20 weeks of gestation and intracranial haemorrhage (ICH) represent the most severe complication: approximately 20% the NAIT with ICH lead to either death or neurological sequel. In Caucasians, 97% of NAIT are caused by HPA-1a or HPA-5b alloantibodies whereas in Asians NAIT is mostly linked to the HPA-4 system. Screening for HPA-1a and HPA 5b parental phenotype represents the first step for early detection and treatment of NAIT, but despite several attempts, no monoclonal antibody specific for HPA-5 had been described. The discrete and conformational nature of HPA-5b epitope (which differs from the HPA-5a allele by a single amino acid substitution) and the very low level of expression of GPIa/IIa (800 - 2800 copies per platelet) have probably contributed to the failure to isolate an HPA-5b specific antibody. To overcome those difficulties, we isolated the peripheral blood lymphocytes from a woman with high titre of anti -HPA-5b alloantibodies. Purified B cells were then immortalized through an optimized combination of CD40-mediated activation and EBV-infection. After 3 weeks in appropriate culture conditions, supernatants were tested with MAIPA against HPA-5b<sup>+</sup> human platelets. Cells from positive wells were cloned by limiting dilution, and a clone secreting an IgG specific for HPA-5b was established (clone F2H11). Antigen specificity was confirmed both with MAIPA and by flow cytometry. Determination of HPA-5 phenotype of 250 healthy blood donors by ELISA using antibody F2H11 showed a very high correlation with genotyping methods (PCRSSP). In conclusion, through a remarkably efficient protocol for human B cells immortalization and cloning, we have generated the first HPA-5b-specific monoclonal antibody that will permit to carry out rapid and accurate phenotyping of pregnant women and to select blood donors.