

## Establishment of anti-horse podoplanin monoclonal antibody using Cell-Based Immunization and Screening (CBIS) method

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**Purpose:** Podoplanin is expressed in normal tissues including renal podocytes and lymphatic endothelial cells. To investigate the expression and function of horse podoplanin (horPDPN), sensitive and specific mAbs against horPDPN are necessary. In this study, we aimed to develop useful anti-horPDPN mAbs for many applications such as flow cytometry (FCM), western blot (WB), and immunohistochemistry (IHC).

**Methods:** We employed a conventional immunization method using synthetic peptides or Cell-Based Immunization and Screening (CBIS) method using horPDPN-expressed mammalian cells for producing anti-horPDPN mAbs. Anti-horPDPN mAbs were screened using enzyme-linked immunosorbent assay or FCM. Established anti-horPDPN mAbs were characterized using FCM, WB, and IHC.

**Results:** We developed two anti-horPDPN mAbs, PMab-202 using the peptide immunization and PMab-219 using CBIS method. PMab-202 reacted with horPDPN in FCM and WB, but did not stain horPDPN in IHC. In contrast, PMab-219 detected horPDPN in not only FCM and WB, but also IHC.

**Conclusion:** We have successfully established mouse anti-horPDPN mAbs, PMab-202 and PMab-219. PMab-219 is applicable for FCM, WB, and IHC analyses. CBIS method could be more advantageous to establish immunohistochemistry-applicable mAbs for elucidating the pathophysiological function of horPDPN.