## Establishment of a novel anti-CD20 monoclonal antibody C<sub>20</sub>Mab-11 using the Cell-Based Immunization and Screening (CBIS) method for the detection of B cells in many applications

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**Purpose**: CD20 is one of B-lymphocyte antigens, and is known as an effective target for B cell lymphomas. Although anti-CD20 monoclonal antibody (mAb) drugs have brought significant survival benefits to B cell lymphoma patients, some patients have shown no clinical response in a second treatment of anti-CD20 mAbs; therefore, more effective treatment of B cell lymphomas should be developed. In this study, we aimed to develop useful anti-CD20 mAbs for treatment or research of B cell lymphoma.

**Methods:** For the anti-CD20 mAb development, we used Cell-Based Immunization and Screening (CBIS) method. CD20-overexpressed LN229 cells (LN229/CD20) were used for immunization. The screening of hybridomas was performed by flow cytometry (FCM) using CD20-overexpressed CHO cells.

**Results:** We used 8 Balb/c mice for the hybridoma development, and obtained 18 anti-CD20 mAbs. Of those clones,  $C_{20}$ Mab-11 (IgM, kappa) was shown to be useful for FCM and western blot (WB) for endogenous CD20-expressing cell lines. Furthermore,  $C_{20}$ Mab-11 strongly stained B cells of the lymph follicle via immunohistochemistry (IHC).

**Conclusion:** Using CBIS method, we successfully developed a sensitive and specific anti-CD20 mAb  $C_{20}$ Mab-11, which is useful for FCM, WB, and IHC.