

Validation data for Anti-mCD20-mIgG2a

<https://www.invivogen.com/anti-mcd20-migg2a-invivofit>

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Version 21C25NJ

Anti-mCD20-mIgG2a InvivoFit™ is a mouse anti-mouse monoclonal antibody (mAb) featuring the variable region of the previously described anti-mCD20 18B12 clone. Using recombinant technology, the original 18B12 murine IgG1 constant region has been replaced with a murine IgG2a format which mediates potent cytotoxic functions. The cell surface staining capacity of Anti-mCD20-mIgG2a InvivoFit™ has been confirmed with HEK293 cells stably expressing mCD20 (HEK-mCD20) (Figure 1). The *in vivo* depletion capacity of Anti-mCD20-mIgG2a InvivoFit™ has been confirmed (Figure 2).

Validation of Anti-mCD20-mIgG2a InvivoFit™ binding by FACS

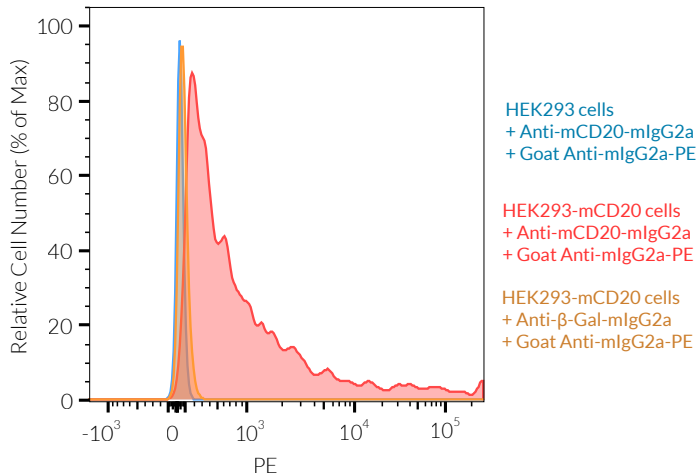


Figure 1: Cell surface staining of murine CD20 using Anti-mCD20-mIgG2a InvivoFit™ mAb.

~5x10⁵ HEK293 (parental) or HEK-mCD20 cells were incubated with 2 μg of Anti-mCD20-mIgG2a InvivoFit™ mAb or an isotype control for 1h at 4°C. Cells were then washed and incubated with 0.25 μg of goat anti-mIgG2a coupled to PE for 1h at 4°C. Cell surface staining was analyzed by flow cytometry.

Validation of *in vivo* B cell depletion using Anti-mCD20-mIgG2a InvivoFit™

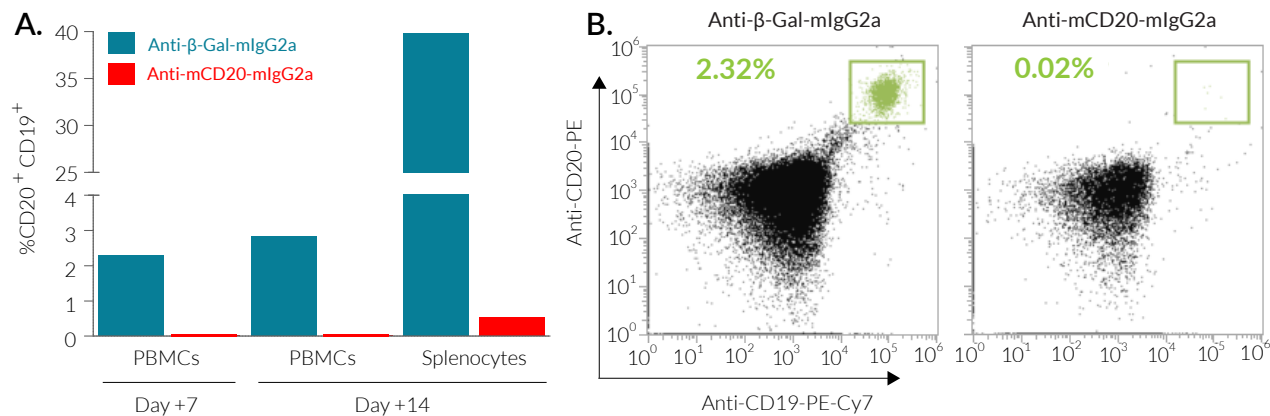


Figure 2: Analysis of CD19⁺ regulatory T cell population in mice upon injection of Anti-mCD20-mIgG2a InvivoFit™ mAb.

SWR/Jmice were injected intraperitoneally (IP) at day 0, +2, +4, and +7 with 200 μg of the depleting Anti-mCD20-mIgG2a InvivoFit™ mAb or Mouse IgG2a control. At day +7 and +14, peripheral blood monocytes (PBMCs) and splenocytes were stained with the following conjugated antibodies: Anti-CD20-PE (clone SA271G2), Anti-CD19-PeCy7 (clone 6D5), according to standard procedures. Cell surface staining was analyzed by flow cytometry. (A) Frequency of CD20⁺CD19⁺ cells among PBMCs and splenocytes at day+7 and day+14. (B) Representative FACS staining of PBMCs at day +7.

TECHNICAL SUPPORT

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