Validation data for Anti-mCD8-mIgG2a

https://www.invivogen.com/anti-mcd8-migg2a-invivofit

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Version 20K10-NJ

Anti-mCD8-mIgG2a InvivoFit™ is a recombinant mouse monoclonal antibody (mAb) designed for *in vivo* depletion in mice. This mAb features the variable region of the previously described anti-mCD8 (YTS 169.4 clone, rat IgG2b κ) mAb and a murine IgG2a constant region. The binding capacity of Anti-mCD8-mIgG2a InvivoFit™ to the murine CD8 (mCD8) antigen has been confirmed using cell surface staining with EL4 cells stably expressing mCD8 (EL4-mCD8) **(Figure 1)**. The *in vivo* depletion of peripheral blood CD8+ T cells using Anti-mCD8-mIgG2a InvivoFit™ has been confirmed **(Figure 2)**.

Validation of Anti-mCD8-mIgG2a binding by FACS

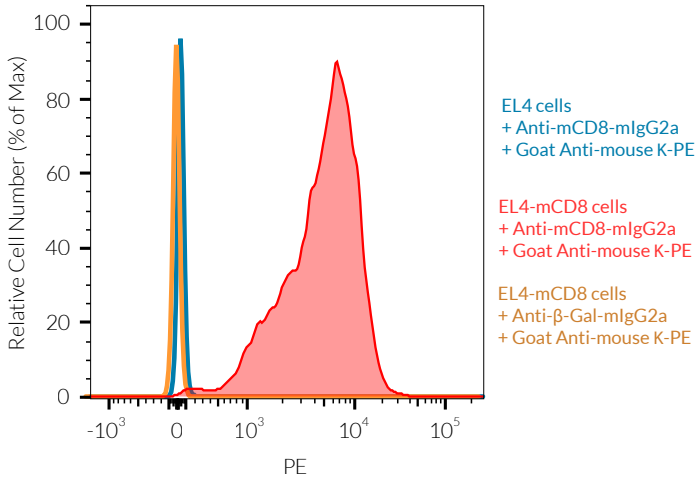

Figure 1: Cell surface staining of murine CD8 using Anti-mCD8-mIgG2a InvivoFit™ mAb.
~5x10^5 EL4 (parental) or EL4-mCD8 cells were incubated with 2 µg of Anti-mCD8-mIgG2a InvivoFit™ mAb or an isotype control for 1h at 4°C. Cells were then washed and incubated with 0.25 µg of goat anti-mouse κ-light chain coupled to PE for 1h at 4°C. Cell surface staining was analyzed by flow cytometry.

Validation of *in vivo* CD8+ T cell depletion using Anti-mCD8-mIgG2a InvivoFit™

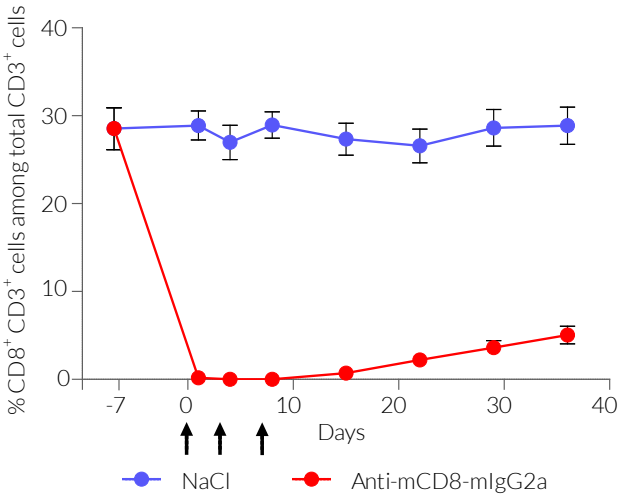

Figure 2: Analysis of CD8+ T cell population in mice peripheral blood upon injection of Anti-mCD8-mIgG2a InvivoFit™ mAb.
Blood samples (20 µl) from SWR/J mice aged 10 weeks were harvested 1 week prior to a series of intraperitoneal (IP) injections with the depleting Anti-mCD8-mIgG2a InvivoFit™ mAb (200 µg) or NaCl (control), and 24h after each IP injection. IP injections were performed at day 0, +3, and +7 (arrows). Thereafter, blood samples were harvested once a week. To assess the kinetics of the *in vivo* depletion of CD8+ T cells, peripheral blood was stained with the following conjugated antibodies: Anti-CD45-Pe-Cy7 (clone 30-F11), Anti-CD3e-PE (clone 145-2C11), Anti-CD4-FITC (clone RMA-4), and Anti-CD8-APC (clone H35-17.2) according to standard procedures. Cell surface staining was analyzed by flow cytometry. Data are represented as the frequency of CD8+ T cells among the total CD3+ T cell population.

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