

# Validation data for Anti-mCD4-mIgG2a

<https://www.invivogen.com/anti-mcd4-migg2a-invivoft>

For research use only

Version 20K10-NJ

Anti-mCD4-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-mCD4 (GK1.5 clone, rat IgG2b κ) mAb and a murine IgG2a constant region. The binding capacity of Anti-mCD4-mIgG2a InvivoFit™ to the murine CD4 (mCD4) antigen has been confirmed using cell surface staining with Jurkat cells stably expressing mCD4 (Jurkat-mCD4) (Figure 1). The *in vivo* depletion of peripheral blood CD4<sup>+</sup> T cells using Anti-mCD4-mIgG2a InvivoFit™ has been confirmed (Figure 2).

## Validation of Anti-mCD4-mIgG2a binding by FACS

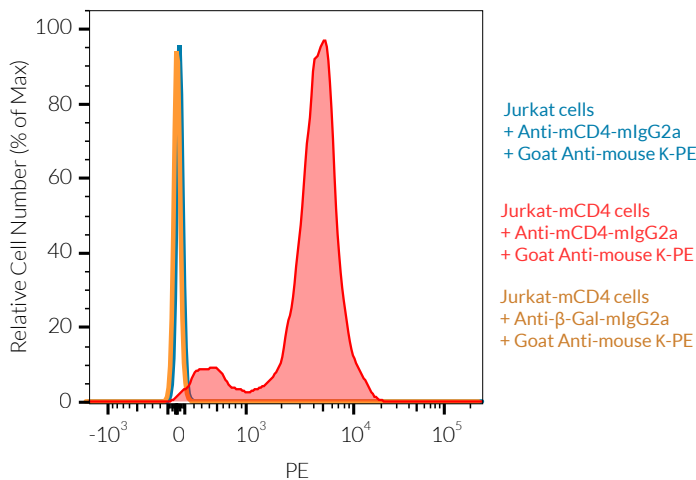


Figure 1: Cell surface staining of murine CD4 using Anti-mCD4-mIgG2a InvivoFit™ mAb.

~5x10<sup>5</sup> Jurkat (parental) or Jurkat-mCD4 cells were incubated with 2 μg of Anti-mCD4-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* CD4<sup>+</sup> T cell depletion using Anti-mCD4-mIgG2a InvivoFit™

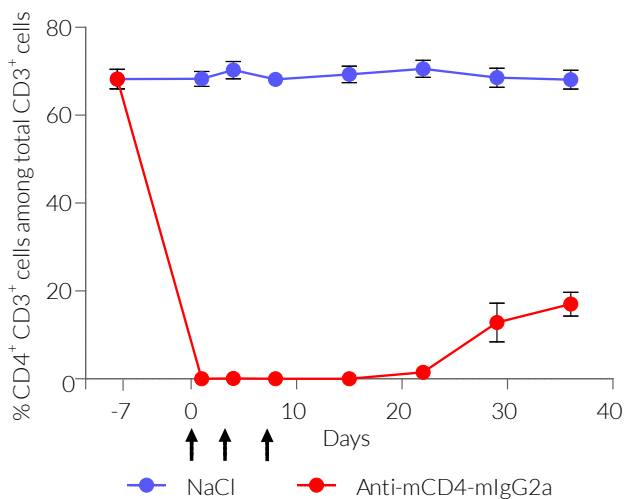


Figure 2: Analysis of CD4<sup>+</sup> T cell population in mice peripheral blood upon injection of Anti-mCD4-mIgG2a InvivoFit™ mAb.

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-mCD4-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 CD4<sup>+</sup> 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 CD4<sup>+</sup> T cells among the total CD3<sup>+</sup> T cell population.

### TECHNICAL SUPPORT

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