

**Table 3: Staining panel for detection of IFN- $\gamma$  by CD4<sup>+</sup> and CD8<sup>+</sup> T cells**

Stain	Purpose
Viability Dye eFluor 506, CD45-PE-Cy7, CD8-FITC, CD4-PE-Cy5, IFN $\gamma$ -PE	Core staining panel to identify live IFN- $\gamma$ producing cells in the CD4 and CD8 cell gates.
FITC-labeled beads or FITC-CD4-labeled splenocytes	Single positive control for compensation
PE-Cy7-labeled beads or PE-Cy7-CD4-labeled splenocytes	Single positive control for compensation
PE-Cy5-labeled beads or PE-Cy5-CD4-labeled splenocytes	Single positive control for compensation
Viability Dye-labeled splenocytes (unstained splenocytes mixed 1:1 with heat-killed splenocytes)	Single positive control for compensation
Unstained splenocytes or beads	Control for compensation
Viability Dye eFluor 506, CD45-PE-Cy7, CD8-FITC, CD4-PE-Cy5	FMO control #1: Used to set gate for IFN $\gamma$ <sup>+</sup> cells.