

Flow Cytometric 4-Color human T cell Panel

Assay robustness

Aim

Establish reliability of assay

Experimental approach

Staining of human peripheral blood mononuclear cells (hPBMCs) with T cell antibody panel:

CD3, CD4, CD8 and dead stain (Near-Infra Red, NIR)

Assay Conditions

- Three donors
- Three staining replicates per donor
- Independent staining on three separate days

Cell subsets of interest

CD molecule matrix & gating hierarchy (4 colors)

- Cytotoxic T cells (NIR⁻) CD3-FITC⁺ CD8-PerCP⁺
- Helper T cells (NIR⁻) CD3-FITC⁺ CD4-Alexa Fluor700⁺

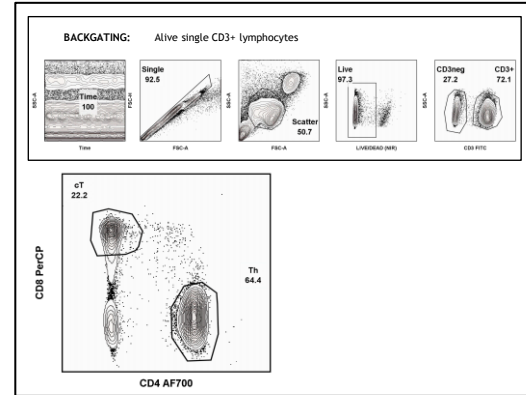
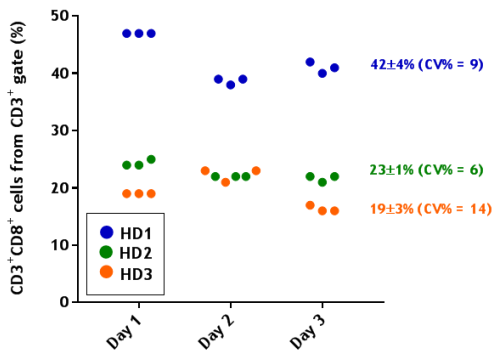
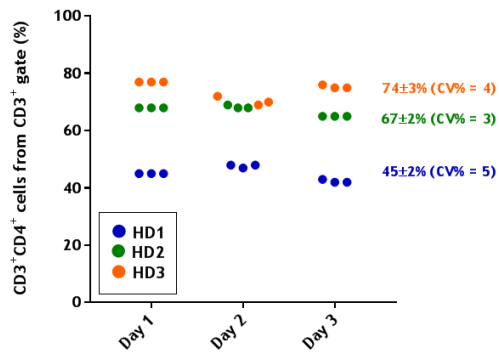


Figure 1. Four-color flow cytometric analysis of CD4 and CD8 T cell frequencies of the CD3-positive population on hPBMCs. Two-parameter dot plot show the correlated expression patterns of CD4 versus CD8 for backgated live single CD3+ lymphocytes. BD LSR Fortessa™-SORP system.



47.1 ± 0.3	38.7 ± 0.5	41.0 ± 1.0
24.1 ± 0.3	22.0 ± 0.2	21.5 ± 0.5
19.0 ± 0.2	22.2 ± 1.3	16.2 ± 0.8

Average ± standard deviation of triplicate measurements for donor samples analyzed on day 1, 2 and 3. Cumulative Z-scores were determined [-2.0;1.0] and calculated according to the formula $(Z_i = \frac{x_i - \bar{x}_{total\ days}}{S_{total\ days}})$.



76.8 ± 0.2	70.5 ± 1.8	75.5 ± 0.7
68.1 ± 0.4	68.4 ± 0.2	64.5 ± 0
45.1 ± 0.2	47.8 ± 0.5	42.4 ± 0.2

Average ± standard deviation of triplicate measurements for donor samples analyzed on day 1, 2 and 3. Cumulative Z-scores were determined [-1.1;2.0] and calculated according to the formula $(Z_i = \frac{x_i - \bar{x}_{total\ days}}{S_{total\ days}})$.

Figure 2. Frequency of cytotoxic T cells and helper T cells. Scatter plot of cytotoxic T cell (left) and helper T cell (right) subset frequency for all replicate measurements for donors across independent days of analysis. The inter-day (figure) and intra-day (table) average values and standard deviations for donor triplicates are shown.

