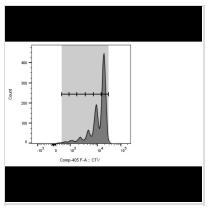

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Proliferation assay

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ABSTRACT

Assessing the impact of drugs and treatments on natural killer (NK) cell viability and expansion.

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1 PBMCs or enriched NK cells (see *Isolating NK cells from human blood products*) are labeled with a permanent amine-reactive dye (CellTrace Violet Proliferation Kit, Cat. No:C34557, Thermo Fisher) according to the manufacturer's instructions.

2 Cells are exposed to various experimental conditions, then harvested after 7 days of culture.

3 *Optional:* If required, cells can be stained for apoptotic markers (FITC Annexin V, Cat. No: 556419, BD Biosciences) according to the manufacturer's instructions prior to antibody staining.

4 Cells are stained with a dead cell marker (LIVE/DEAD NEAR-IR, Cat. No: L34976, Thermo Fisher), PE-CY7 conjugated anti-CD56 (HCD56, BioLegend), and PE-CF594 conjugated anti-CD3 (UCHT1, BD BioSciences).

5 The samples are run on a flow cytometer (LSRII, BD Biosciences) and analyzed using FlowJo software (Tree Star Inc., RRID:SCR_008520).

The proliferation of live (dead cell marker- and annexin V-) NK cells (CD56+/CD3-) and T cells (CD56-/CD3+) is assessed by dilution of the dye.

Relative numbers of live NK cells and T cells for each treatment condition are obtained by running the flow cytometer at a constant flow rate and analyzing the number of each cell type obtained within a specified time limit e.g. 60s.

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