PHOSPHOFLOW CYTOMETRIC ASSAY

Accurate evaluation of signaling molecules at the cellular level

About PHOSPHOFLOW CYTOMETRY

The phosphorylation of numerous proteins is involved in cellular signaling cascades. Flow cytometry, coupled with fluorescently-labeled antibodies specific to various phosphorylated proteins, enables the precise detection of these crucial molecules at the single-cell level. Moreover, this phosphoflow cytometric assay allows for simultaneous examination of phosphoproteins within distinct cell subsets present in samples containing multiple cell types. Compared to traditional assays like Western Blots that only provide an overview of global protein status within seemingly homogeneous populations, the phosphoflow cytometric assay offers a more powerful and sensitive approach.

How does THE Assay work?

Phosphoflow cytometric assays can specifically detect the state of protein phosphorylation at the cellular level. Our assays are optimized for multiple cell types including immune cells. A general work flow is as follows:

- Treated and control cells or tissue/blood samples are processed and stained with viability dye and cell surface markers antibodies.
- cells are fixed and permeabilized.
- cells are then stained with the antibodies for intracellular phosphoproteins.
- cells are then acquired on a flow cytometer.

Assay BENEFITS

- **Superior specificity:**
  - ✓ Delivers greater specificity than other common technologies like Western blot, potentially revealing heterogeneity in a seemingly homogeneous cell sample.
  - ✓ Measure phosphorylation at a single cell level.
  - ✓ Provides data for mechanistic insights.
- **Flexibility:** easily automated for use in routine compound screening
- **Simultaneous detection:** detect several targets (phosphoproteins and other markers) in the same cell type or in distinct cell subsets in a heterogeneous cell sample.
- **Standardized platform:** 96-well plate format using Cytoflex S flow cytometer.
- **Use of specialized reagents:** Assays are optimized for accurate and reproducible data.

Sample requirements

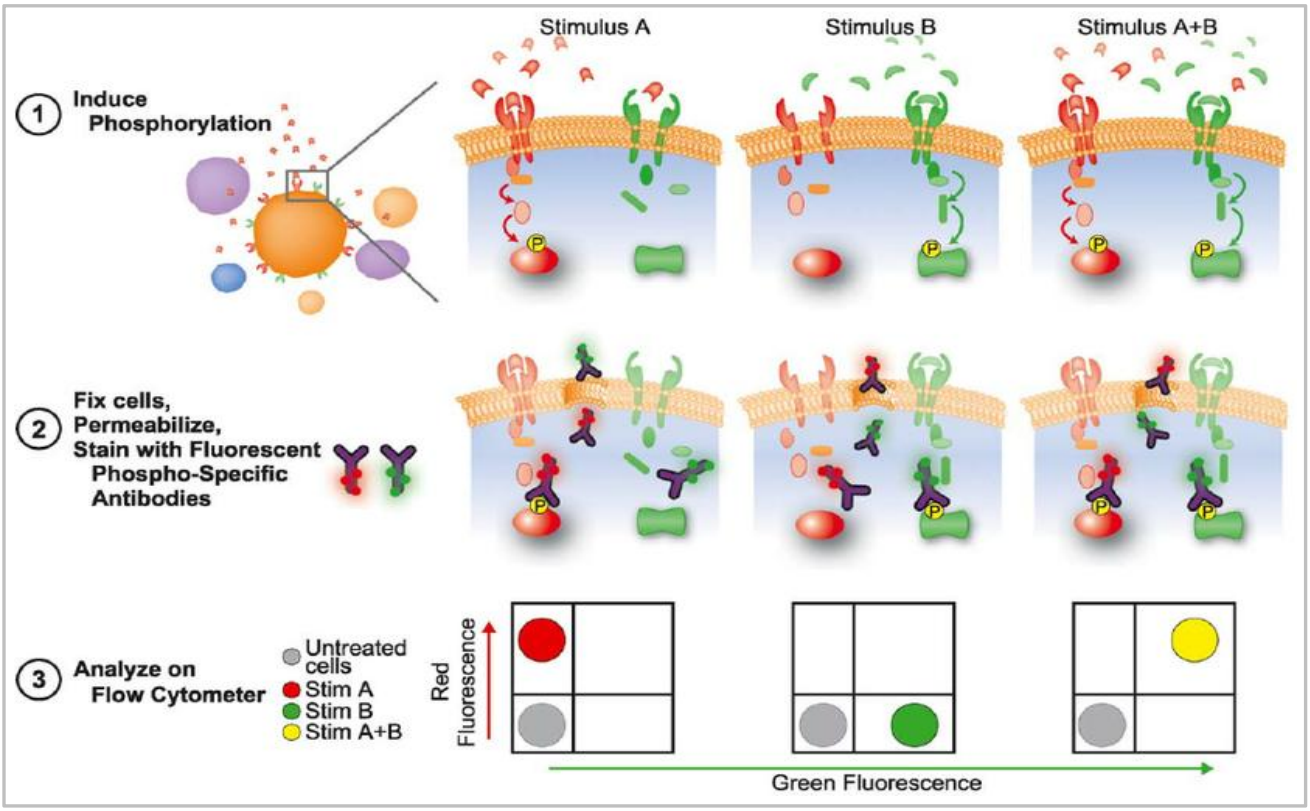
Minuscule samples

Compatibility: works with a variety of sample types such as cultured cells, whole blood, PBMCs, isolated tissue cells, etc.

Service features

- **High throughput:** 96-well format
- **Simultaneous quantitation:** detect phosphoproteins in multiple cell populations in the same sample and/or multiple phosphoproteins in the same cell type
- **Highly reproducible**
- **State-of-art platforms:** Cytoflex S flow cytometers
- **Large inventory of phosphoprotein specific antibodies**
- **Timely data delivery:** 1-4 weeks or sooner, upon receiving test samples
- **20+ years of experience:** Extensive expert data analysis and interpretation, high quality scientific and technical support

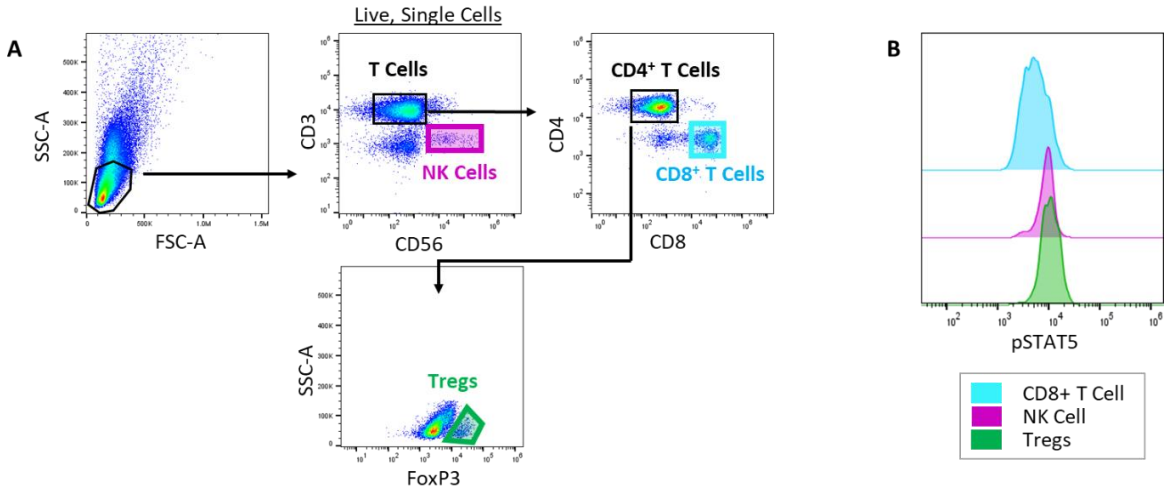
Key ASSAY STEPS



PicoImmune Data examples

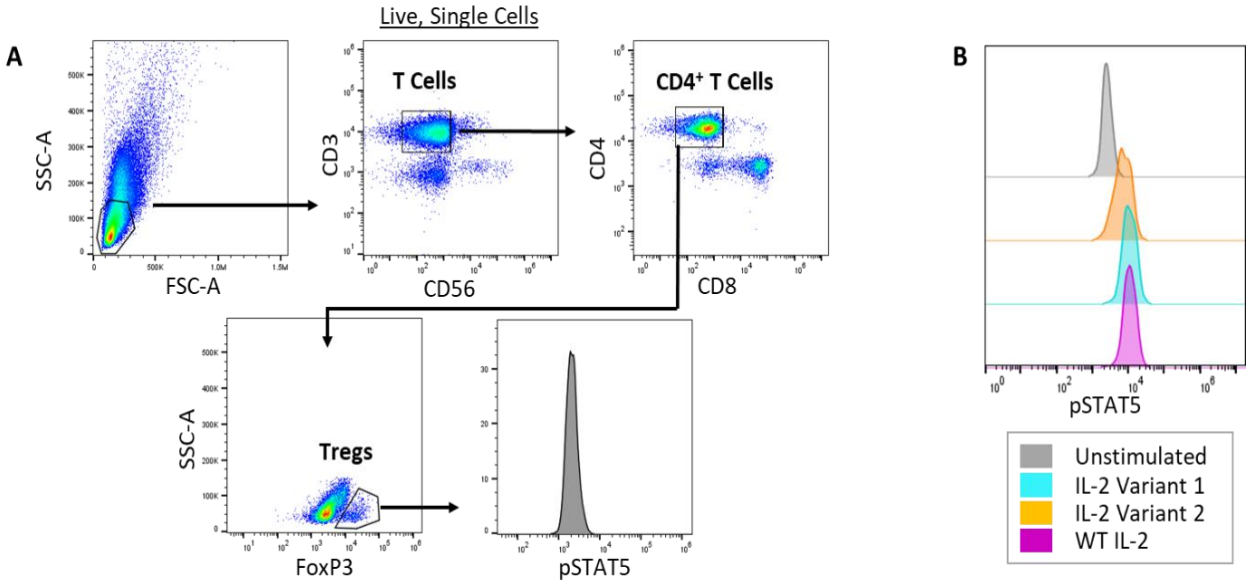
Example 1. Multidimensional analyses with phospho-specific flow cytometry.

(A) Gating strategy to identify Tregs and examine STAT5 phosphorylation in a human PBMC samples. Human PBMCs were treated with 60 ng/mL of wildtype (WT), Variant 1 and Variant 2 recombinant IL-2. The cells were then stained with fluorescently-conjugated antibodies against CD3, CD56, CD4, CD8, FoxP3 and phosphorylated STAT5 (pSTAT5). (B) The level of STAT5 phosphorylation in Tregs in response to a fixed concentration of different IL-2 variants was analyzed.



Example 2. Evaluation of pSTAT5 in multiple cell subsets following IL-2 treatment.

(A) Gating strategy to identify Natural Killer (NK) cells, CD8+ T cells and Tregs in a PBMC sample. Normal human PBMCs were treated with 60 ng/mL of wildtype (WT) recombinant IL-2. The cells were then stained with fluorescently-conjugated antibodies against CD3, CD56, CD4, CD8, FoxP3 and phosphorylated STAT5 (pSTAT5). (B) The level of STAT5 phosphorylation in Natural Killer (NK) cells, CD8+ T cells and Tregs in response to WT IL-2 was analyzed.



Example 3. The effect of IL-2 treatment on STAT5 phosphorylation in Tregs.

Human PBMCs were treated with varying concentrations of wildtype (WT), Variant 1 and Variant 2 recombinant IL-2 for 2 h. The cells were then stained with appropriate fluorophore-conjugated antibodies and acquired on the Cytoflex S cytometer. The mean fluorescence intensity (MFI) of phosphorylated STAT5 (pSTAT5) was determined at each concentration.