Bruker's Workflow and Recommendations for Flow Sort Stains

The following workflow has been used in generating singlecell functional data and serves as a guideline for flow sort staining with the Bruker system.

In this Technical Note we outline:

- · Workflow recommendations for stain combinations
- · Bruker's standard protocol and flow sorting protocol



Prep, Run, Analyze

Bruker's Standard Protocol and Recommended Flow Sorting Protocol a) 1. Sample Enrichment b) 1. Sample Enrichment Thaw Cells with IL-2 Thaw Cells with IL-2 O/N Recovery O/N Recovery Stain Cells with Flow Enrich for CD8+T Cells **Sorting Surface** Marker Stains* CD8+T Cell BV711 Enrich for CD4+T Cells **BV786** CD4+T Cell BV711 + BV786 PE-Cy7 2. Cell Stimulation Stim on Plate with PMA/ Ionomycin PMA/IONO Stim on plate Flow Sort Cells 1.5 hrs Stimulated T Cell Stim on Plate with PMA/ Ionomycin PMA/IONO Stim on plate Add Cell Add Cell Stain 405 Stain 405 1.5 hrs to CD4+ to CD8+ Stimulated T Cell 10 min CD8+T Cells 4. Load Cells Add Cell Add Cell Stain 405 Stain 405 to CD4** to CD8+* Load onto IsoLight CD8+T Cell 4. Load Cells *Flow stain used per manufacturer's directions. Flow stains must be labeled in IsoSpeak as red. Load onto IsoLight **Label as Violet in IsoSpeak. For more information on labeling in IsoSpeak, please refer to the IsoSpeak Manual, chapter 4. It is critical to use an Bruker validated stain after flow sorting. Figure 1 | a) Standard Protocol. Sample enrichment, cell stimulation, cell staining, load cells. b) Flow Sorting Protocol. Flow sorting, cell stimulation, cell staining, load cells.

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Workflow Recommendations for Stain **Combinations**

Bruker has validated flow sort staining for CD4+ and CD8+ T cells on the IsoLight. Users can now flow sort cells prior to loading into their IsoLight system (Figure 1). The following workflow is recommended:

- 1. Thaw PBMCs. Thaw cryopreserved PBMCs and resuspend cells with complete RPMI and IL-2.
- 2. Recover PBMCs. Incubate cells overnight.
- 3. Stain PBMCs. Using the recommended flow stains, as shown in Figure 1, stain the cell subset of interest CD4+ and or CD8+ T cells. Use flow stains per manufacturer's directions.
- 4. Flow sort. Sort the cells using standard flow sorting

- 5. Stimulate cells. Collect sorted cell subset of CD4+ and or CD8+ T cells. Stimulate the cells on plate for 1.5 hours with Cell Activation Cocktail (PMA and Ionomycin).
- **6. Thaw IsoCode chips.** Allow IsoCode chips to thaw for 30-60 minutes prior to use. Set up all liquid reagents in instrument
- 7. Stain CD4+/CD8+ T cells. Using Bruker's cell stain 405, stain each cell subset. It is critical to use an Bruker validated stain after flow sorting.
- **8. Load cells onto chips.** Resuspend cells using complete RPMI to a density of 1 x 106 cells/mL and load 30 µL of cell suspension onto IsoCode chip.
- 9. Load all chips onto instrument and run experiment.
- 10. Analyze data using IsoSpeak. In IsoSpeak, label flow sort stains as red and cell stain 405 as violet.