TECHNICAL NOTE

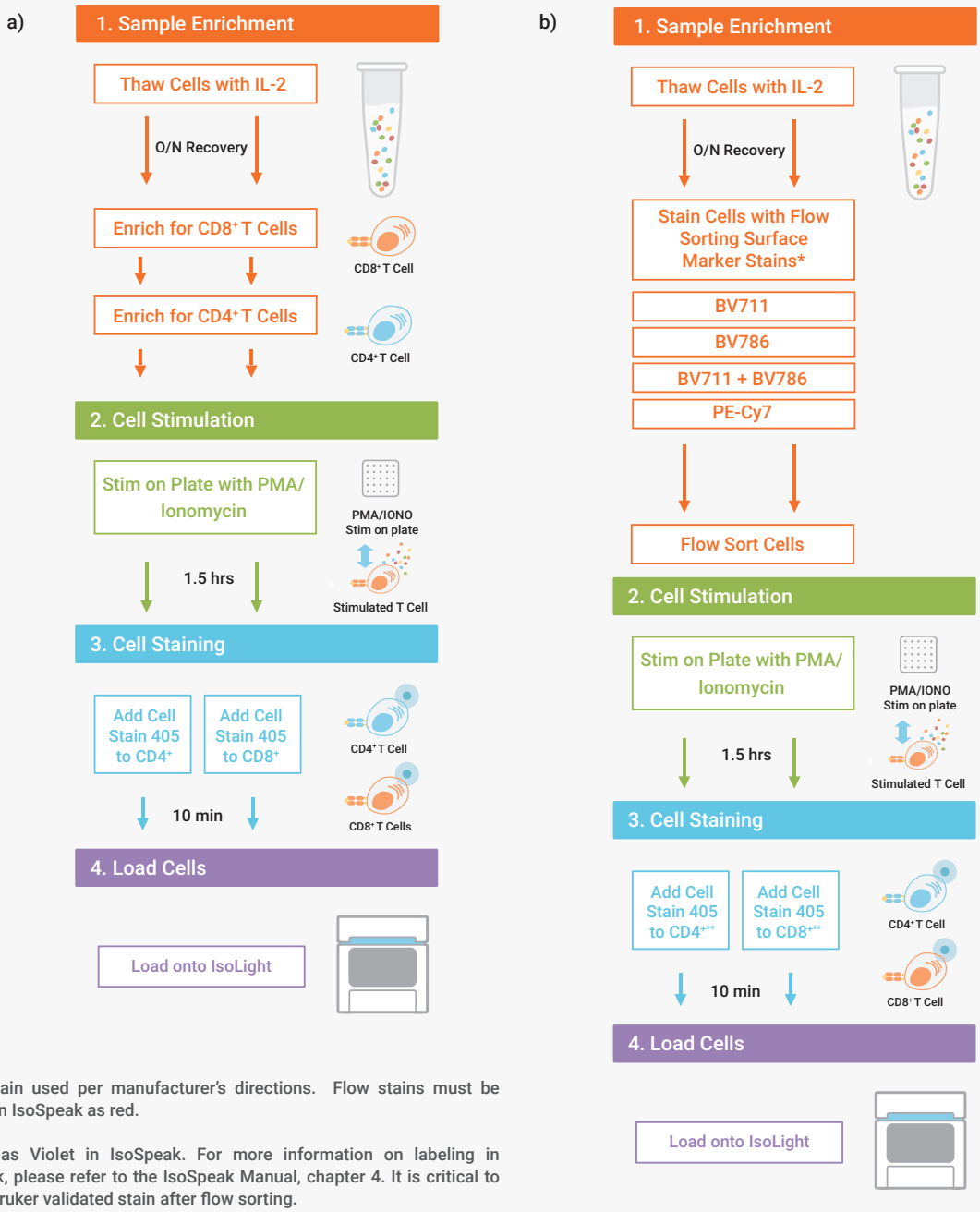
Bruker's Workflow and Recommendations for Flow Sort Stains

The following workflow has been used in generating single-cell 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

Bruker's Standard Protocol and Recommended Flow Sorting Protocol



*Flow stain used per manufacturer's directions. Flow stains must be labeled in IsoSpeak as red.

**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.

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 protocol.

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 1×10^6 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.