TECHNICAL NOTE

Cell Staining Guidelines for IsoCode Assays

Staining requirements for your IsoCode kit, cell type of interest and application protocol

In this Technical Note we outline:

- Importance of Cell Staining for IsoCode Assays
- Staining Requirements for IsoCode Kits and Compatible Cell Types
- Cell Stain Descriptions for Bruker's Application Protocols

Importance of Cell Staining for IsoCode Assays

Cell staining is a critical component of sample preparation for IsoCode assays. Staining ensures cell detection during imaging that occurs within our instruments and that the minimum required number of cells of a given phenotype are present on the chip. Refer to Table 1 for staining requirements for your IsoCode kit and cell type of interest. Please consult with your FAS if your cell type of interest does not appear in the table or for any questions.

Note: *Stains and staining procedures not approved by Bruker will require validation prior to use.*

Staining Requirements for IsoCode Kits and Compatible Cell Types

Application	Kit	Cell Type	Validated Cell Stain(s)
Single-Cell Secretome 	**Human Adaptive Immune**	Bispecific T Cells	Cell Stain 405
		CAR-T Cells	Alexa Fluor® 647-CD4 & Alexa Fluor® 647-CD8
		CD154+ T Cells	Cell Stain 405 and either Alexa Fluor® 647-CD4 or Alexa Fluor® 647-CD8†
		iPSC-derived Astrocytes	Cell Stain 405 & FITC CD49f
		Monocyte-Derived Dendritic Cells (MoDCs) iPSC-derived Astrocytes	CellTrace™ Far Red Cell Proliferation Kit
		PBMCs	3 Options*: (1) Cell Stain 405 (2) Cell Stain 405 & APC-CD45RO (3) Alexa Fluor® 647-CD4 & Alexa Fluor® 647-CD8
		PBMCs (Stimulated with PMA & Ionomycin)	Cell Stain 405
		TCR-T Cells	Cell Stain 405
		Tumor-Associated Endothelial Cells	Cell Stain 405
		Tumor-Associated Fibroblasts	Cell Stain 405
	Human Inflammation	Bispecific T Cells	Cell Stain 405
		CAR-T Cells	Alexa Fluor® 647-CD4 & Alexa Fluor® 647-CD8
		CD154+ T Cells	Cell Stain 405 and either Alexa Fluor® 647-CD4 or Alexa Fluor® 647-CD8†
		iPSC-derived Astrocytes	Cell Stain 405 & FITC CD49f
		Monocyte-Derived Dendritic Cells (MoDCs)	CellTrace™ Far Red Cell Proliferation Kit
		PBMCs	3 Options*: (1) Cell Stain 405 (2) Cell Stain 405 & APC-CD45RO (3) Alexa Fluor® 647-CD4 & Alexa Fluor® 647-CD8
		PBMCs (Stimulated with PMA & Ionomycin)	Cell Stain 405
		TCR-T Cells	Cell Stain 405
		Tumor-Associated Endothelial Cells	Cell Stain 405
		Tumor-Associated Fibroblasts	Cell Stain 405

Table 1: Cell staining guidelines for Bruker's IsoCode assays

Staining Requirements for IsoCode Kits and Compatible Cell Types (continued)

Application	Kit	Cell Type	Validated Cell Stain(s)
Single-Cell Secretome (continued) 	**Human Innate Immune**	Monocyte-Derived Microglia-like Cells (MDMi)	Cell Stain 405
		Monocytes	Cell Stain 405
		Myeloid Stem Cells	Cell Stain 405
	Human Natural Killer	NK Cells	Cell Stain 405
	Mouse Adaptive Immune	Astrocytes	CellTrace™ Far Red Cell Proliferation Kit
		Bone Marrow (BM) Cells	Cell Stain 405
		Mouse T Cells	2 Options‡: (1) Cell Stain 405 (2) Cell Stain 405 & either Alexa Fluor® 647 anti-mouse CD4 or Alexa Fluor® 647 anti-mouse CD8a
	Mouse Innate Immune	Monocytes	Cell Stain 405
	Non-Human Primate (NHP) Adaptive Immune	PBMCs	Cell Stain 405
Single-Cell Signaling 	**Human Tumor Signaling**	HeLa Cells	Cell Stain 405

* Dependent on experimental design. See IsoCode Single-Cell Adaptive Immune: Human PBMC Protocol for full details or consult with your FAS.

† Dependent on experimental design. See IsoCode Single-Cell Adaptive Immune: Human CD154+ T Cell Protocol for full details or consult with your FAS.

‡ Dependent on experimental design. See IsoCode Single-Cell Adaptive Immune: Mouse T-cells Protocol for full details or consult with your FAS.

Note: *CodePlex Secretome assays do not require cell staining.*

Table 1: Cell staining guidelines for Bruker's IsoCode assays (continued)

Cell Stain Descriptions for Bruker's Application Protocols

- **Alexa Fluor® 647-CD4 & Alexa Fluor® 647-CD8 [CD3/CD28 Human PBMC (option) and CAR-T Protocols]:** Alexa Fluor® CD8 and CD4 stains are an insightful method for specifically staining T cells. Using this stain as a part of your workflow can ensure the detection of quality cells that only possess the surface marker of interest.
- **APC-CD45RO [CD3/CD28 Human PBMC Protocol (option)]:** APC-CD45RO stain, in conjunction with the use of Cell Stain 405, is an inclusive method for staining T cell subsets. Using these stains as a part of your workflow can help in discerning proteomic profile differences between a mixed population of CD45RO+ memory T cells and presumptive CD45RO naïve T cells.
- **CellTrace™ Far Red (Human Monocyte-Derived Dendritic Cell and Mouse Astrocyte Protocols):** CellTrace™ Far Red is a reliable method for non-specifically staining cells. Using this stain as a part of your workflow can ensure the detection of quality cells regardless of stimulation type or surface marker presence.
- **Cell Stain 405 and either Alexa Fluor® 647-CD4 or Alexa Fluor® 647-CD8 (CD154+ Protocol):** Cell Stain 405 is a reliable method for non-specifically staining cells. The additional inclusion of AF647 CD8 or CD4 stains are an insightful method for specifically staining T cells.
- **Cell Stain 405 and CD4/CD8 Stain (Mouse T Cell Protocol):** Cell Stain 405 is a reliable method for non-specifically staining cells. The additional use of CD8 and/or CD4 specific surface marker staining is recommended for TILs or T cells in a debris rich environment where enrichment alone does not guarantee a high purity sample.
- **Cell Stain 405 (All other protocols):** Cell Stain 405 is a reliable method for non-specifically staining cells. Using this stain as a part of your workflow can ensure the detection of quality cells regardless of stimulation type or surface marker presence.