FACT SHEET

CyTOF Inflammatory Response Assay

POWERFUL SINGLE-METHOD ALTERNATIVE TO FLOW CYTOMETRY AND LIGAND BINDING ASSAYS

Aliri BIOANALYSIS

Cytometry by Time-Of-Flight (CyTOF), is an advanced bioanalysis technique that combines aspects of flow cytometry with mass spectrometry to analyze single cells with high precision.

Commonly applied to immune cell profiling, immunophenotyping, cancer immunotherapy, single-cell functional analysis, and infectious disease research, CyTOF tracks cytokine levels and immune dynamics over time to yield critical data about the mechanism of action, efficacy, safety, and immunomodulatory effects of a drug in real time.

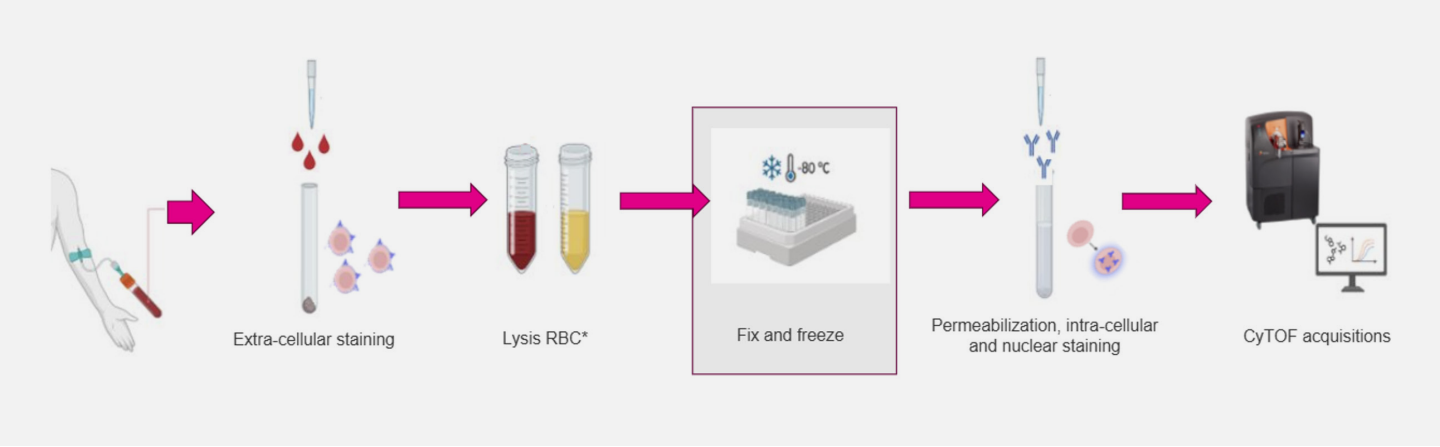
Aliri's skilled team of scientists have over five years of experience using this multi-omics/single cell method on various matrices to support cellular immunophenotyping and inflammatory response monitoring, as it provides sponsors a more detailed understanding of complex immune cell populations including their function and activation.

WHAT IS CYTOF?

- Utilizes heavy metal isotope-labeled antibodies for cellular marker detection
- Cells are ionized, and metal ions are analyzed using time-of-flight mass spectrometry, which determines which markers are present on each cell

TECHNICAL ADVANTAGES OF CYTOF OVER FLOW CYTOMETRY

- **High-dimensional analysis:** Analyze 40+ markers per cell in a single run.
- **Minimal background noise:** Cells don't naturally contain heavy metal isotopes, resulting in clean signals.
- **Single-cell resolution:** Ideal for profiling rare cell populations and distinct cell states.
- **No autofluorescence:** Eliminates background signal common in fluorescence-based methods.
- **Clear, distinct signals:** Each metal tag has a unique atomic mass, avoiding spectral overlap.
- **Consistent signal intensity:** Reduced variability compared to fluorescence.
- **Deep immune profiling:** Enables detailed phenotyping of T cells, B cells, monocytes, NK cells, and their subsets.
- **Room temperature stability:** 72-hours of stability removing the need for cold-chain shipping.
- **No need for PBMC processing:** This ensures more consistent results due to no operator-to-operator variation and is more cost effective



ALIRI'S NEXT GENERATION INFLAMMATORY RESPONSE ASSAY:

- Analyzes 46 markers off the shelf
  - Cell markers
  - Functional markers
  - Intracellular cytokines
- Inflammatory data tied directly to cell type - no need for two different methods using Flow Cytometry and kit-based Ligand Binding Assays
- Single blood draw – lower sample volume reduces patient morbidity. This method is highly compatible with pediatric and rare disease indications, as well as select oncology trials.
- Standard assay can be customized and validated for specific markers and indications

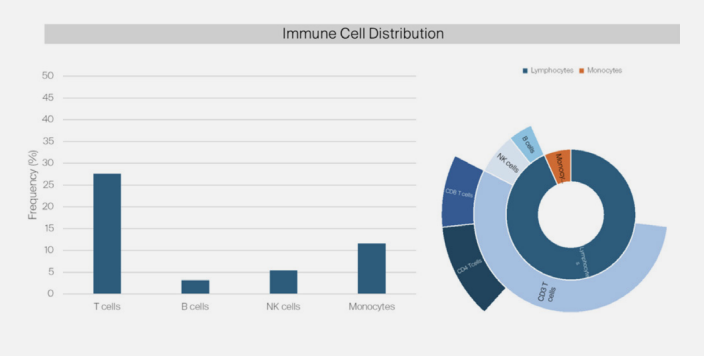
Summary Count Example

		COUNT	% INTACT LIVE CELLS	PARENTS	% PARENTS
Intact Live Cells		77511	Time Point	All events	88.7
Lymphocytes		35887	46.3	Intact Live Cells	46.3
	CD3 T Cells	21388	27.6	Lymphocytes	59.6
	IL-6	4021	5.2	CD3 T Cells	18.8

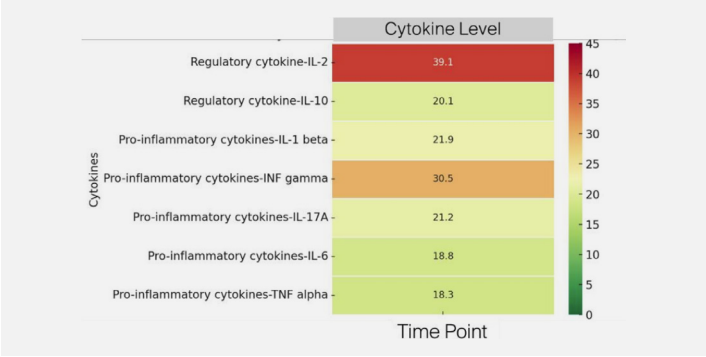
Staining Assessment Example

MARKER	POSITIVE	NEGATIVE	GRADE	POS MEDIAN	NEG MEDIAN
CD3	CD4	CD20	M	120.1	48.9
CD19	B	CD4	M	111.2	20.1
CD14	Monocyte Classical	CD4	G	406.6	75.1
CD27	CD8 Naïve + CM	CD8 Terminal effector	G	356.9	42.3
CD16	Late NKs	CD8 Naïve	VG	764.5	80.8
CD45RA	CD4 Naïve	CD4 Effector memory	G	567.9	12.7

Immune Cell Categories Distribution Example



Inflammatory Profile Example



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