

Mass Cytometry

Q&A

Q&A based on the LabRoots presentation A Multi-Omic Approach to Detection and Characterization of Viral Pathogens and Their Impact on the Immune System

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As researchers around the world mount an aggressive and sustained response to the COVID-19 pandemic, it is important to build on past successes and harness insights from current efforts to better detect and characterize pathogens, identify treatments and prepare for future outbreaks.

The best-in-class immune profiling and monitoring capabilities of Fluidigm mass cytometry, powered by CyTOF® technology, have been applied to research efforts currently underway. The Maxpar® Direct™ Immune Profiling System is the first complete sample-to-answer solution for high-dimensional immune profiling of human PBMC and whole blood. Designed as a simple, single-tube workflow, the system brings together CyTOF technology with the Helios™ system, a dry 30-marker antibody panel and automated Maxpar Pathsetter™ software to easily count 37 immune cell populations.

Find out more or **contact** your local Field Applications Scientist (FAS).

If new antibodies are added to the Maxpar Direct panel, can the automated software option still be used?

Yes. An important feature of the Maxpar Pathsetter software is that it allows for easy addition of new antibodies to the assay panel backbone. The software includes automated analysis for the base 30-marker panel. Markers can be added to the Maxpar® Direct™ Immune Profiling Assay™ Pathsetter model for your specific needs. You can ask new questions about different cell populations or cell function. In a recent example, a lab added several new antibodies to the panel for COVID-19 studies. Please reach out to your local FAS through techsupport@fluidigm.com to find out more.

What is a Smart Tube, and what is the protocol for freezing whole blood samples?

The Smart Tube System and the Smart Tube Proteomic Stabilizer are used to preserve and stabilize whole blood for later analysis with mass cytometry. Use of Smart Tubes for the Maxpar Direct Immune Profiling Assay has not been evaluated.

For the Maxpar Direct Assay, we have demonstrated storing stained whole blood samples at –80 °C for up to 120 days. Please find more information in the **Application Note: FLDM-00089**.

Is there evidence that the fixatives in the Maxpar Direct staining protocol inactivate COVID-19, particularly by formalin fixation or fixation and permeabilization (fix/perm) steps?

Currently, we do not have data on SARS-CoV-2 inactivation by paraformaldehyde (PFA) or fix/perm fixation.

For more information:

- “Handling and processing blood specimens from patients with COVID-19 for safe studies on cell phenotype and cytokine storm”: onlinelibrary.wiley.com/doi/10.1002/cyto.a.24009
- “Virological assessment of hospitalized patients with COVID-19”: nature.com/articles/s41586-020-2196-x
- ISAC Biosafety Committee regarding COVID-19: isac-net.org/news/news.asp?id=497501

Are any COVID-19 projects using metal isotope-labeled antibodies with mass cytometry? Or is fluorescence labeling more suitable in this case?

Several recent COVID-19 publications include the use of mass cytometry for deep analysis of the immune response¹⁻⁵, and we expect to see the number of these projects grow. **Leng** et al. published a mass cytometry study of patients with COVID-19 associated pneumonia. Patients were treated with mesenchymal stem cells (MSCs).

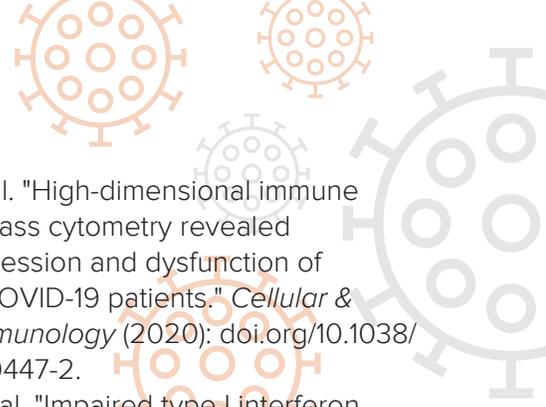
This study involved MSC transplantation with ACE2⁻ cells. Immune response was monitored by using a 36-marker mass cytometry panel. A notable advantage of CyTOF technology is the ability to test larger marker numbers in a single panel, compared to studies that use fewer markers and are run over multiple tubes instead of one single tube. Metal labeling of antibodies can also support application of the different techniques required to perform intracellular staining in addition to examining surface markers. This, along with a high degree of plexity, allows for much easier panel design using mass cytometry.

What is the turnaround time for mass cytometry?

Turnaround time depends on which assay is being run and whether you have access to a system. If you have a Helios system or have access to one, you can process, stain and analyze samples in a single afternoon. The system provides very high throughput, allowing several samples to be quickly run at once. If you do not have access to Helios and send samples to Therapeutic Insights Services, turnaround time is very project-specific. Turnaround time can be as fast as 1–2 weeks from the time of sample receipt. The project time frame can be discussed in more detail with a Therapeutic Insights Services representative.

What is the turnaround time to get results from Therapeutic Insights Services?

Turnaround time is very project-specific. It depends largely on the number of custom conjugations, staining requirements, the number of biological samples, the number events (for mass cytometry), the number of regions of interest (for Imaging Mass Cytometry™) and level of data analysis required. For data acquisition on pre-stained samples and minimal data analysis, turnaround time can be as fast as 1–2 weeks from the time of sample receipt. The project time frame can be discussed in more detail with a Therapeutic Insights Services representative.



Can I send frozen PBMC samples to Therapeutic Insights Services and have the Maxpar Direct Assay run on them?

Yes, we offer Maxpar Direct Immune Profiling Assay services for frozen PBMC pellets. We can generate a custom quote for mass cytometry projects.

Must all samples be fixed in smart buffer, or a similar buffer, before they are sent to Therapeutic Insights Services for CyTOF analysis?

Use of a Smart Tube system is study-dependent. Sample preparation conditions depend on the purpose of the study, and they can be discussed on a per-project basis with a Therapeutic Insights Services scientist. Please see the Fluidigm website for details on standard sample preparation protocols.

Of note, the Maxpar Direct Immune Profiling Assay has not been validated after preserving blood using Smart Tubes. Smart Tube buffer is typically used for preserving whole blood. It is especially useful for cytokine stimulation on fresh whole blood followed by freezing the tubes in buffer until further testing can be performed with phosphoflow. Because Smart Tube buffer may degrade a number of surface antigens, use of it depends on the purpose of the study. Most studies use cryopreserved PBMC for surface or intracellular cytokine staining (ICS) antigen detection.

References

1. Wang, W. et al. "High-dimensional immune profiling by mass cytometry revealed immunosuppression and dysfunction of immunity in COVID-19 patients." *Cellular & Molecular Immunology* (2020): doi.org/10.1038/s41423-020-0447-2.
2. Hadjadj, J. et al. "Impaired type I interferon activity and exacerbated inflammatory responses in severe COVID-19 patients." *medRxiv* (2020): doi.org/10.1101/2020.04.19.20068015.
3. Ouyang, Y. et al. "Down-regulated gene expression spectrum and immune responses changed during the disease progression in COVID-19 patients." *Clinical Infectious Diseases* (2020): ciaa462.
4. Zhang, Y. et al. "Inflammatory Response Cells During Acute Respiratory Distress Syndrome in Patients with Coronavirus Disease 2019 (COVID-19)." *Annals of Internal Medicine* (2020): doi:10.7326/L20-0227.
5. Leng, Z. et al. "Transplantation of ACE2-mesenchymal stem cells improves the outcome of patients with COVID-19 pneumonia." *Aging and Disease* 11 (2020): 216–228.

Learn more about COVID-19 and Fluidigm innovation at fluidigm.com/covid-19

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