

# CyTOF XT

## Frequently Asked Questions

### Hardware

#### **What is the footprint of CyTOF XT?**

The footprint of CyTOF® XT is 0.7 m<sup>2</sup> (7.5 square feet). The footprint is smaller than Helios™, and no additional space is needed for an external chiller. The chiller is now integrated into the CyTOF XT™ system.

#### **What is the argon consumption of CyTOF XT?**

CyTOF XT uses 15 L/min of argon when plasma is running. This is 25% less than Helios. For more information on argon specifications, refer to our CyTOF XT System Site Requirements Guide (FLDM-00360).

#### **What is the mass channel range of CyTOF XT?**

A total of 135 mass channels are detected on CyTOF XT, ranging from 75 to 209 atomic mass units (amu), the same mass range as Helios.

#### **Are earlier-generation mass cytometer consumable parts compatible for use on CyTOF XT (for example, cones, torch, nebulizer, and injectors)?**

No. All CyTOF XT parts have been optimized for use with CyTOF XT. For more information, refer to the CyTOF XT User Guide (FLDM-00254).

#### **What is the maximum number of sample tubes that can be accommodated in the Autosampler Module carousel on CyTOF XT?**

The chilled carousel has 12 positions for 5 mL tubes and 1 position for a 15 mL tube.

## What types of tubes can be loaded in the Autosampler Module carousel on CyTOF XT?

The CyTOF XT chilled carousel component of the Autosampler Module can accommodate 12 x 75 mm 5 mL round-bottom polypropylene tubes for carousel positions 1–12, and 20 x 76 mm 15 mL conical-bottom skirted tubes for carousel positions 13–15.


## What volume ranges can be selected for sample resuspension and acquisition from each compatible sample tube type on CyTOF XT using the Autosampler Module?

The sample resuspension and acquisition volume ranges are 150  $\mu$ L to 4 mL for the 5 mL sample tube types and 150  $\mu$ L to 12 mL for the 15 mL sample tube types. To ensure that air is not aspirated by the probe line:

- When acquiring suspended samples, add an extra 100  $\mu$ L of sample to each tube.
- When acquiring pelleted samples, the pellet should remain in a residual volume of approximately 100  $\mu$ L.

## Can the carousel and plasma be observed while the instrument is running?

Yes. Cameras are available to monitor the current carousel tube position below the probe as well as the plasma conditions. The Autosampler camera can be used to observe sample resuspension, mixing, stirring, and acquisition. The plasma camera displays the plasma.

For more information, click the Help icon  to access the integrated help guide for CyTOF Software v8.0 Help for CyTOF XT, which is also available as a printable PDF (FLDM-00045).

## Workflow and Maintenance

### How long is the startup time to plasma stabilization on CyTOF XT?

After plasma startup, allow 30 min for the components of the instrument to reach optimal temperature and stabilize.

### Can CyTOF XT resuspend and mix samples and add EQ Six and/or EQ Four Element Calibration Beads?

Yes. The CyTOF XT Autosampler Module accommodates 2 sample formats: cell suspension and cell pellets. Set acquisition volume for suspension samples, resuspension volume for pelleted samples, and addition of beads in the batch acquisition protocol settings for each sample. Adjust mixing and stirring settings in the Template Editor.

## How often do I need to load fresh EQ Six or EQ Four Element Calibration Beads to the Autosampler Module carousel on CyTOF XT?

We recommend preparing a fresh tube of EQ™ Six Element Calibration Beads (EQ6 beads) that are thoroughly shaken and vortexed daily. It is necessary to add a sufficient volume of EQ6 beads for tuning and for completing batch acquisitions. EQ Four Element Calibration Beads (EQ4 beads) can be used on CyTOF XT for normalization and are recommended for use with samples processed with the Maxpar® Direct™ Immune Profiling Assay™.

## What is the estimated time for daily QC (tuning and bead sensitivity testing) on CyTOF XT?


Tuning and bead sensitivity testing are now consolidated into one step using EQ6 beads on CyTOF XT. The approximately 10 min, one-step automated daily quality control on CyTOF XT is quicker than the 25–30 min required for Helios instrument tuning and bead sensitivity testing.

## Can I import my Helios acquisition template into CyTOF Software v8.0?

Yes. In CyTOF Software v8.0 for CyTOF XT, mass channels from Helios templates (.tem) can be imported into an acquisition template in the Template Editor window. Other settings selected in the CyTOF XT acquisition template (for example, mixing and stirring) are set to default values.

## Does CyTOF XT run automated wash cycles between samples?

Yes. Pre-wash cycles of different durations and cleaning solutions can be selected in the batch acquisition protocol. You can program each cycle to the necessary level of washing before running each sample.

For more information, click the Help icon  to access the integrated help guide for CyTOF Software v8.0 Help for CyTOF XT, which is also available as a printable PDF (FLDM-00045).

## What is a batch acquisition?

Batch acquisition allows the user to load up to 13 sample tubes into the Autosampler Module carousel and initiate the run. The automated system acquires the samples in the set in the order selected in the acquisition protocol. Each sample in the batch can be acquired using customized acquisition settings and a unique template, if desired.

## **Can I add more samples to the carousel after a batch acquisition is initiated?**

Yes. The instrument design and software support the addition of walk-up samples. Users can add new sample tubes to available positions in the chilled carousel during the queued batch acquisition. Sample tubes can be added after the acquisition of earlier samples is completed and as carousel locations become available.

## **What happens when a clog is detected? If the clog cannot be removed automatically, what happens to the remaining sample in the lines?**

Two pressure sensors trigger the automated clog detection and removal routine and determine the location of the clog. The system attempts to remove the clog by pulsing forcefully and flushing the lines and nebulizer. Depending on the location of the clog and whether Recover Sample was selected in the acquisition template, the sample is returned to the sample tube or discarded.

## **Do I need to manually clean the instrument at the end of the day before switching off plasma?**

We recommend cleaning the nebulizer daily with 10% Contrad® 100/Decon™ 90 or switching to another clean nebulizer. For detailed instructions, see the CyTOF XT User Guide (FLDM-00254).

## **Does the instrument run an automated cleaning procedure at the end of the batch before switching off plasma?**

An automatic cleaning procedure is performed at the end of a batch acquisition. If the checkbox to perform an extended clean is selected in the acquisition protocol, a Heavy wash cycle is performed. If the Run extended clean checkbox is not selected in the acquisition protocol, a Medium wash cycle is performed.

## **Is there an automated plasma shutdown procedure on CyTOF XT?**

Yes. A checkbox for shutting down plasma can be selected in the batch acquisition protocol settings.

## **How often should routine maintenance of CyTOF XT instrument parts be performed?**

The nebulizer should be cleaned daily. CyTOF XT parts (for example, the spray chamber, injector, and cones) should be cleaned weekly or after 115 hours of operation. The torch should be cleaned every 3 months. For detailed instructions on cleaning procedures, see the CyTOF XT User Guide (FLDM-00254).


## Sample Types and Acquisition

### What cell types have been successfully acquired on CyTOF XT?

A variety of samples have been acquired on the CyTOF XT system, including fresh and frozen human whole blood and peripheral blood mononuclear cells, mouse and human cell lines, and dissociated solid tissues from mice (intestine and spleen).

### What are the recommendations for acquiring samples from dissociated tissues on CyTOF XT?

Acquisition of dissociated tissue samples can be optimized by following our recommendations for sample preparation as well as maintenance before, during, and after batch acquisition. If necessary, manual intervention can also be performed to remove unresolved clogs.

For more information, click the Help icon  to access the integrated help guide for CyTOF Software v8.0 Help for CyTOF XT, which is also available as a printable PDF (FLDM-00045).

### What format of cell samples is recommended for acquisition in the chilled carousel?

Samples in 5 mL carousel tubes can be prepared as cell suspensions or as cell pellets. Samples in 15 mL carousel tubes should always be prepared as cell suspensions. If the 15 mL sample tube size is being used, we recommend acquiring this sample first.

### What is the recommended sample concentration for CyTOF XT?

The recommended sample concentration is 1 million cells/mL.

### What is the sample flow rate on CyTOF XT?

The flow rate is 30  $\mu$ L/min.

## Reagents

### What solution is used for resuspending and acquiring samples on CyTOF XT?

Maxpar Cell Acquisition Solution Plus for CyTOF XT (PN 201244) is an improved high-ionic-strength solution intended for sample preparation and sample acquisition on CyTOF XT systems.

## What is the difference between EQ Six (EQ6) and EQ Four (EQ4) Element Calibration Beads?

EQ6 beads contain  $^{89}\text{Y}$ ,  $^{115}\text{In}$ ,  $^{140}\text{Ce}$ ,  $^{159}\text{Tb}$ ,  $^{175}\text{Lu}$ , and  $^{209}\text{Bi}$ . EQ4 beads contain  $^{140}\text{Ce}$ ,  $^{151/153}\text{Eu}$ ,  $^{165}\text{Ho}$ , and  $^{175}\text{Lu}$ . EQ6 beads are used for tuning on CyTOF XT by calculating dual counts per bead for an accurate tuning representation of cellular events. EQ6 and EQ4 beads are used for data normalization. Either EQ bead can be selected from the carousel to be added to a sample as defined by settings in the Template Editor and the Acquisition Protocol. EQ4 beads are recommended for use with the Maxpar Direct Immune Profiling Assay for compatibility with Maxpar Pathsetter™ analysis.

## Are all suspension mass cytometry Maxpar antibodies and Cell-ID reagents compatible for use on CyTOF XT?

Yes. All Maxpar antibodies and Cell-ID™ suspension mass cytometry reagents are compatible for use on CyTOF XT.

## Are all Maxpar solutions compatible for use on CyTOF XT?

Maxpar Water, Maxpar Cell Acquisition Solution Plus for CyTOF XT, and Maxpar Washing Solution for CyTOF XT are sold in specialized bottles to fit in the bottle reservoir station on CyTOF XT. For ordering information, see the Product Overview chapter in the CyTOF XT User Guide (FLDM-00254).

**NOTE** Maxpar Tuning Solution is not for use on CyTOF XT.

## Are there updated staining protocols for use with CyTOF XT?

Yes. All Fluidigm staining protocols, including the Maxpar Direct Immune Profiling Assay and Cell-ID 20-Plex Pd Barcoding Kit User Guides, have been updated to include sample preparation details for CyTOF XT acquisitions, which includes the use of Maxpar Cell Acquisition Solution Plus and EQ6 beads.

## Is the Maxpar Direct Immune Profiling Assay compatible with CyTOF XT? Can these samples be analyzed with Maxpar Pathsetter software when acquired on CyTOF XT?


Yes. The Maxpar Direct Immune Profiling Assay is compatible with CyTOF XT. Maxpar Direct Immune Profiling Assay data acquired on CyTOF XT can be analyzed with Maxpar Pathsetter software if samples are acquired with the designated acquisition template, EQ4 beads, and the EQ4-MDIPA bead setting.

## Can I acquire barcoded samples on CyTOF XT?

Yes. Samples barcoded with the Cell-ID 20-Plex Pd Barcoding Kit or stained using CD45 antibody-based live-cell barcoding are both supported on the CyTOF XT system. The 15 mL tube is designed to accommodate a sample volume of up to 12 mL at 1 million cells/mL. Alternatively, barcoded samples with more than 12 million cells can be aliquoted into multiple 5 mL tubes to be resuspended and acquired from a pellet format.

## Software and Data Analysis

### Is there an online help guide for CyTOF XT Software v8.0?


Yes. A built-in, searchable help guide is updated both online and remotely to ensure current information. Click the Help icon  to access the integrated guide or download it for user convenience. The help guide is also available in PDF form as the CyTOF Software v8.0 Help for CyTOF XT, FLDM-00045.

### Can CyTOF Software v8.0 for CyTOF XT be run as stand-alone software for data processing?

Yes. CyTOF Software v8.0 for CyTOF XT is available for downloading from the Fluidigm website for stand-alone use.

### What are the output file types for samples acquired on CyTOF XT?

Numerous output files are available on CyTOF XT. Raw data is compressed to linear mode data (LMD) files and processed to FCS 3.1 files. CyTOF Software v8.0 for CyTOF XT automatically generates raw and normalized FCS files that are ready immediately after acquisition.

For more information, click the Help icon  to access the integrated help guide for CyTOF Software v8.0 Help for CyTOF XT, which is also available as a printable PDF (FLDM-00045).

### How is normalization different on CyTOF XT?

EQ6 or EQ4 beads may be used for data normalization on CyTOF XT. EQ6 beads are used for data normalization in real time during sample acquisition on CyTOF XT. EQ6 beads contain 6 metal isotopes from <sup>89</sup>Y to <sup>209</sup>Bi to robustly normalize mass channels spanning the broad range of detection on CyTOF XT. EQ4 beads are recommended for use with Maxpar Direct Immune Profiling Assay on CyTOF XT to maintain compatibility with Maxpar Pathsetter automated analysis. Updates to the CyTOF Software v8.0 improve upon the built-in sample standardization, using EQ calibration beads to minimize technical variability. An improved algorithm better identifies the EQ calibration beads on the fly, producing analyzable data immediately after acquisition.

## Can samples be debarcoded using the CyTOF Software v8.0?

Yes. CyTOF Software v8.0 includes a Debarcoder in the Data Processing tab for 20-plex sample multiplexing capabilities. For multi-tube barcoded batch samples, the data must be normalized and concatenated before debarcoding.

## What data analysis software platforms are recommended for use with CyTOF XT files?

Normalized FCS file output (\_Processed.fcs, FCS 3.1) is generated automatically during acquisition. Most third-party software tools can be used to analyze FCS 3.1 files.

## Relevant Documents

Document name	Document number
CyTOF XT System Site Requirements Guide	FLDM-00360
CyTOF XT User Guide	FLDM-00254
CyTOF Software v8.0 Help for CyTOF XT	FLDM-00045

### For technical support visit [techsupport.fluidigm.com](https://techsupport.fluidigm.com).

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