New Sample Acquisition Protocols

A new protocol for staining on Helios and CyTOF 2-to-Helios Upgrade

Introduction

A change to the Helios™ staining and acquisition protocols for routine use has been developed to improve cell integrity and staining quality. These protocols together enable higher intensities and tighter CVs for signals from metal labeled-cells, maximizing results from precious samples. The new WB Injector (PN 107950) in conjunction with the Maxpar® Cell Acquisition Solution (CAS) (PN 201237) improves data quality and enables you to maximize results from your precious samples. These sample acquisition protocols are recommended for Helios and Helios upgrade customers.

Protocol Updates: Acquisition Using the WB Injector and CAS

Updates to the tuning and acquisition protocols on Helios consist of the following:

- Implementation of the newly designed WB Injector on the instrument. The WB Injector replaces the HT injector on Helios and CyTOF® 2-to-Helios upgrades.

- Implementation of Cell Acquisition Solution (CAS) for sample acquisition on Helios and CyTOF 2-to-Helios upgrades, with WB Injector ONLY. CAS replaces DIW for sample acquisition.

Installing the WB Injector

Replacing the HT Injector

IMPORTANT Please use Maxpar Cell Acquisition Solution on Helios when using the WB Injector.

IMPORTANT The injector should be fully dry prior to inserting.

NOTE The WB Injector is etched with the letters WB near the base of the injector.

1 Install the WB Injector by pushing until it is fully inserted. Turn the injector counterclockwise to tighten the Injector Sealer Cap.

IMPORTANT When installing the WB Injector for the first time, confirm that the injector is 1.5–2 mm from the end of the inner portion of the torch.

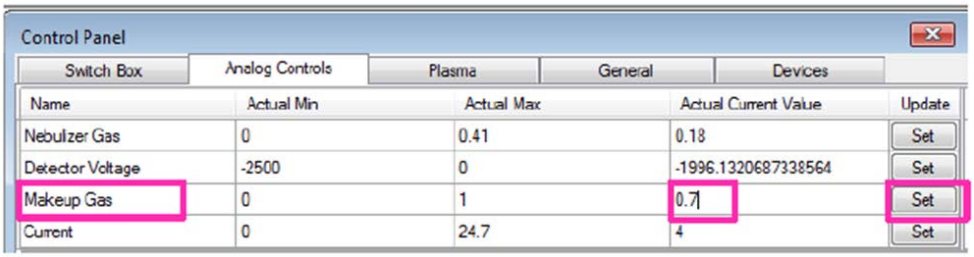
IMPORTANT Refer to the Helios User Guide (PN 400250) for detailed instructions on installing the WB injector after weekly cleaning.

Adjusting the Makeup Gas with the WB Injector

To use the WB Injector in your instrument you must change the makeup gas flow value from the setting used for the HT injector.

1 Click **Control Panel** in the menu panel of the CyTOF Software, and then click **Analog Controls**.

2 Set the Actual Current Value for Makeup Gas to +0.2 L/min higher than current value, and then click **Set**. In the example below the Actual Current Value was set to 0.5 L/min for the HT injector, so the Actual Current Value for the WB Injector is set to 0.7 L/min.



3 Run a Full Protocol tuning prior to running your samples on the system (refer to the Helios User Guide PN 400250).

NOTE CyTOF Tuning Solution (PN 201072) is required for this procedure.

Sample Acquisition

Acquiring with the WB Injector requires 1) conditioning the sample introduction system with CAS prior to sample acquisition and 2) diluting samples in EQ™ Four Element Calibration Beads and CAS.

Instrument Conditioning

Following tuning and the bead sensitivity test, condition the system by running Maxpar Cell Acquisition Solution for 15 minutes prior to acquiring samples.

1 Load the 5 mL tube with 3 mL of CAS onto the Sample Loader.

2 Turn the Sample Loader on by clicking **ON** in the sample introduction controls.

3 Run CAS for 15 minutes.

Sample Preparation

This should be performed following the bead sensitivity test. See Helios User Guide (PN 400250, Chapter 5: Operation).

To maximize data quality, filter samples to reduce clumps and dilute them to optimal concentration before loading them onto the Sample Loader.

1. Prepare the sample at the appropriate concentration in a 1.5 mL or 5 mL tube.
2. Count cells using a hemocytometer.

   NOTE It is strongly recommended that users dilute EQ Beads in CAS and then resuspend samples. The EQ Beads are used as an internal standard for normalization.

3. Vigorously shake or vortex the bottle with EQ Beads. Then dilute the EQ Beads 1/10 in CAS.
4. Resuspend the sample in the diluted EQ Beads (in CAS).
5. Dilute samples to the maximum recommended sample concentration for the cell type but not exceeding 1.0 x 10^6 cells/mL.

   NOTE A higher concentration may result in a higher number of aggregates and lower singlet throughput.

6. Filter each sample with a cell strainer of the appropriate size for the cells in the sample. The sample is now ready for acquisition.

Helios Maintenance

Use of the WB Injector and CAS requires a different maintenance schedule for the injector and cones.

WB Injector Cleaning

Daily (After 8 hours of sample acquisition)

1. Remove the ball joint clamp that secures the Spray Chamber to the WB Injector.
2. Slide the heater off the heater box pins and rest the heater on the upper support pins.
3. Remove the WB Injector by gently pulling and turning until it comes loose from the Torch Assembly.
4. Rinse the WB injector in DIW and dry thoroughly.

Weekly

1. Soak the WB Injector in 10% Contrad®/Decon for 1 hour.
2. Scrub with a brush.
3. Rinse with DIW.
4. Dry thoroughly before reinstalling on instrument.

Sampler and Skimmer-Reducer Cone Cleaning (every 12 hours of sample acquisition)

IMPORTANT Refer to the Helios User Guide (PN 400250) for the detailed procedure.

1. Place sample and skimmer-reducer cones with adapters into the cleaning container.
2. Soak and sonicate in 10% Citranox® (15 minutes maximum).
3. Rinse with DIW.
4. Repeat soak and sonication steps 2 and 3 with DIW three times.
5. Dry thoroughly before reinstalling.

Summary

Improvements to the data acquisition protocols on Helios result in better cell integrity and staining quality. Effective use of the newly designed WB Injector and Maxpar Cell Acquisition Solution can help improve results for Helios and Helios upgrade customers.

For technical support visit techsupport.fluidigm.com. | For general support visit fluidigm.com/support.

North America +1 650 266 6100 | Toll-free (US/CAN): 866 358 4354 | support.northamerica@fluidigm.com **Latin America** +1 650 266 6100 | techsupportlatam@fluidigm.com
Europe/Middle East/Africa/Russia +44 1223 859941 | support.europe@fluidigm.com **China (excluding Hong Kong)** +86 21 3255 8368 | techsupportchina@fluidigm.com
Japan +81 3 3662 2150 | techsupportjapan@fluidigm.com **All other Asian countries/India/Australia** +1 650 266 6100 | techsupportasia@fluidigm.com