ab229130 FirePlex-HT® Imager Setup Kit

For optimizing performance of high-content imagers, for use with FirePlex-HT multiplex immunoassays.

This product is for research use only and is not intended for diagnostic use. Please read entire protocol booklet prior to starting, as protocols are subject to updates.

Table of Contents

1. Background

This protocol contains instructions for setting up a high-content imager (HCI) to acquire FirePlex-HT® multiplex particles. Some imagers may not be able to resolve the particles to a sufficient level for analysis. The Imager Setup Particles are designed to help identify properly resolving HCIs for FirePlex-HT® immunoassays.

2. High-content imager Requirements

Detection	Color Channel	Excitation	Emission
16-bit sCMOS Camera(s)	Green	440–500 nm	500–535 nm
	Yellow	440–500 nm	570–630 nm
	Red	490–560 nm	650–760 nm

3. Precautions

Please read these instructions carefully prior using the equipment.

All components have been formulated and quality control tested to function successfully. Modifications to the components or procedures may result in loss of performance.

4. Storage

Store kit at 4°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt.

5. Materials Supplied

Item	Quantity	Storage Condition
384 Well Imaging Plate and Plate Seal	2 Units	RT
HCI Imager Setup Particles*	1 Unit (1ml)	4°C
*note: particles should be protected from light at all times.		

6. Limitations

– Modifications to the components or procedures may result in loss of performance.

7. Particle Preparation and Acquisition

7.1 Mix the provided vial of HCI Imager Setup Particles well by vortexing.

7.2 Plate 35 µl of the mix to a well of the provided 384-well imaging plate. Load a total of 3 wells onto the plate (e.g. wells A1-C1).

7.3 Seal the plate with the supplied adhesive plate seal.

7.4 Load your plate into your imager.

7.5 Use your imager's plate dimensions definition software to develop a plate coordinates definition for the FirePlex 384-well Imaging plate. Examples from the Molecular Devices ImageXpress Micro and PerkinElmer Opera Phenix are included below for reference. Please consult your manufacturer's instructions for setting plate definitions on your specific model.

ImageXpress Micro

Opera Phenix

Please enter the dimensions of the plate type.

A:	11.22 mm	C:	127.00 mm	E:	114.55 mm
B:	9.61 mm	D:	86.00 mm	F:	77.11 mm

Side:

G: 14.12 mm

H: 1.90 mm

I: 0.64 mm

Scan H & I

Top:

L: 3.63 mm

M: 3.63 mm

Bottom:

O: 2.67 mm
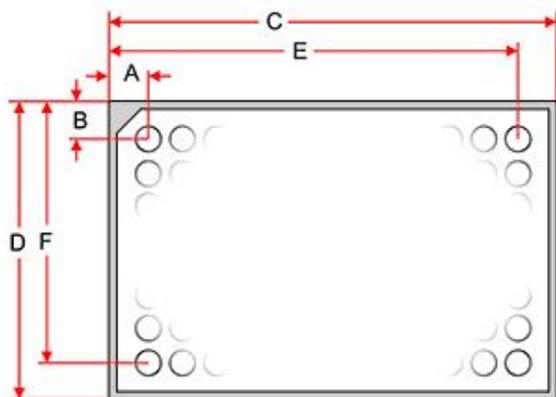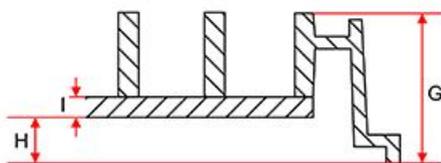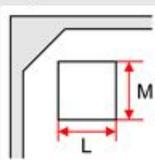
P: 2.67 mm

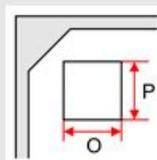**7.6**   Select a 2x2 Binning method coupled with a 4x or 5x magnification objective setting.

7.7   Confirm that the X-Y coordinates for the wells of the plate are accurate by test imaging wells A1, P1, A24 and P24. Start by imaging the particles you plated in Well A1. Use the 4x-5x magnification objective and the **Green fluorescence** excitation and filter set (e.g. **Blue Light excitation** [440-500 nm LED] with **Green emission** [515-535 nm]), and determine an appropriate Z-axis offset that will bring the particles into focus.

7.8   Test image wells A1, P1, A24 and P24 to confirm that the exposure at each position is centered in the well. If wells are significantly off-center (<80% well coverage in the exposure), go back to your imager's plate definition software and adjust any appropriate X-Y coordinates to get the images centered in the wells.

7.9   Return to the A1 well position and adjust the Green fluorescence exposure so that the 3 distinct levels of Green fluorescence present on every particle are visible. The two ends of the particle will have high and medium Green fluorescence, respectively; the center particle region will have low Green fluorescence (see image below).
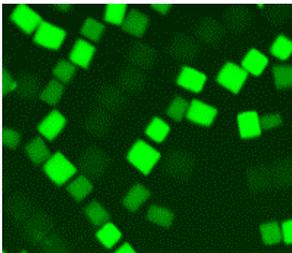

*Note: Exposures should be adjusted such that the particle regions with high Green fluorescence have pixel values well-below the saturation point for the HCI instrument being used (e.g. if your machine has a 65k gray-scale range, target the highest Green fluorescence to be between 15k-20k gray-scale value). Exposure times should be limited to <1 second for this parameter.

7.10 Adjust the **Yellow fluorescence** detection using an appropriate excitation and emission filter set (e.g. **Blue Light excitation** [440-500 nm LED] with **Yellow emission** [570-630 nm]). Yellow fluorescence will primarily be on the ends of the particles (see image below). Locate the brightest Yellow regions and tune the exposure to yield gray-scale values that are about half the saturation max of your machine (e.g. if your machine has a 65k gray-scale range, target the highest Yellow fluorescence to be between 30k-40k gray-scale value).
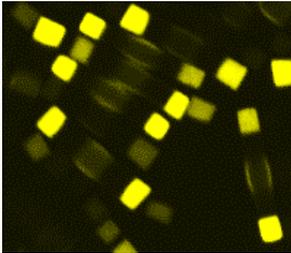
7.11 Adjust the **Red fluorescence** detection using an appropriate excitation and emission filter set (e.g. **Green/Yellow Light excitation** [490-565 nm LED] with **Red emission** [650-760 nm]). Red fluorescence will be focused in the middle section of some of the particles (see image below). Locate the brightest Red regions and tune the exposure to yield gray-scale values that are about half the saturation max of your machine (e.g. if your machine has a 65k gray-scale range, target the highest Red fluorescence to be between 30k-40k gray-scale value).
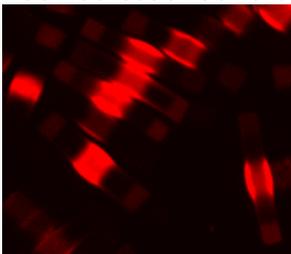
7.12 Ensure that Z-axis offset values that you have work for each fluorescent parameter.

7.13 Acquire a test image of the well being used to tune detector exposure times. All 3 fluorescent images should be below pixel saturation, and are similar to the images below.

Green	Yellow	Red	Merge
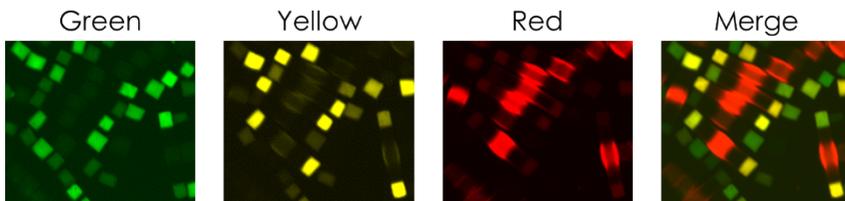
7.14 Acquire and save a full set of images for all 3 wells that contain particles.

7.15 Perform subsequent exposures, adjusting the **Red fluorescence** detection to have robust signal without reaching non-linear signal saturation. For example, top signal values can be adjusted to equal approximately 10%, 50%, and 75% of the saturation value for the Red channel, respectively.

7.16 Export TIFF files and annotate the provided "Imager Acquisition Notes" page (Section 8 of this booklet), recording filter sets used and exposure times employed for the given fluorescent parameters (see Section 8).

7.17 Store the imaging plate at 4°C, protected from light. The same plate can be used for up to 7 days to test imaging parameters on other microscopes.

*Note: In instances where particles have dried out or become too photobleached to be useful, fresh particles can also be plated again in empty wells of the same plate.

8. Imager Acquisition Notes

Date:

User:

8.1 Physical Imager Specifications

Machine (Make/Model):_____

Bit range of camera (e.g. 14-bit, 16-bit): _____

4X Objective make/model: _____

Blue Light Source (Laser or LED, Wavelengths):

_____

Yellow/Green Light Source (Laser or LED, Wavelengths):

_____

Green Fluorescence Filter wavelengths: _____

Yellow Fluorescence Filter wavelengths:

_____

Red Fluorescence Filter wavelengths: _____

8.2 Acquisition Variables

Name of Acquisition File (e.g. Test 1, Test 2, etc.):

_____

Binning Method (1x1, 2x2): _____

8.3 Exposure Times (per well)

Green Channel (ms): _____

Yellow Channel (ms): _____

Red Channel (ms): _____

Technical Support

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259
France
supportscientifique@abcam.com | 01.46.94.62.96
Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154
Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com
Deutsch: 043-501-64-24 | Français: 061-500-05-30
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
ca.technical@abcam.com | 877-749-8807
US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823
China
cn.technical@abcam.com | 400 921 0189 / +86 21 2070 0500
Japan
technical@abcam.co.jp | +81-(0)3-6231-0940
Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450
New Zealand
nz.technical@abcam.com | +64-(0)9-909-7829