

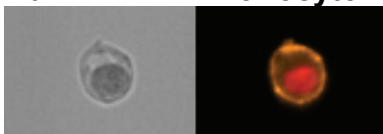
# Sample Preparation Guide



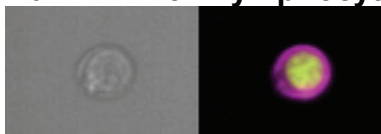
**Experimental Design:** The ImageStream system can quantify the intensity, specific location, and distribution of signals within tens of thousands of cells per sample. The system can perform most any standard flow cytometric assay, but the best applications take advantage of the technology's imaging capabilities to discriminate subtle morphologic or signal distribution changes within individual cells and cell populations.

- Choice of Cell Type:** The cell/particle size should be less than 45 microns in diameter. The system can analyze a wide variety of cell types and applications. Example imagery is shown below:

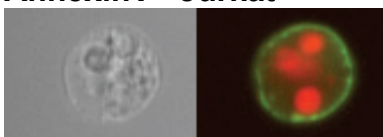
**HuPB CD14+ Monocyte**



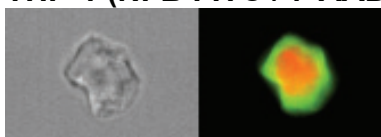
**HuPB CD45+ Lymphocyte**



**AnnexinV+ Jurkat**



**THP-1 (NFB FITC / 7-AAD)**



- Protocols:** In general, any established labeling protocol used for flow cytometry will work with the ImageStream (see *Current Protocols in Cytometry* for general labeling techniques). Stain cells on ice in the presence of azide when possible to reduce non-specific capping of antibody. Use polypropylene tubes, preferably siliconized, to process samples.
- Choice of Fluorochromes:** Choose fluorochromes from the table on **page 4** that are excited by the lasers in your ImageStream (405, 488, 561, 592 and/or 658 nm). Channel 1/9 are always brightfield. SSC imagery may be placed into channel 6 if desired. Dyes with an \* are excited by at least one laser directed to camera 1 and another directed to camera 2. For these dyes, the channel that the dye will appear brightest in depends on the relative laser powers used. Recommended dyes are indicated in boldface.

Please note that this is not a complete list of all of the fluorochromes that will work on the ImageStream. Please consult Amnis before choosing other dyes.

- Controls:** For spectral compensation, it is important to have unlabeled cells as well as cells labeled with a single-color positive control for each fluorochrome used (i.e. FITC only cells, PE only cells, etc.).
- Cell Aggregation:** We strongly advise de-aggregation of clumps as a final step before straining the sample through a 70 micron nylon mesh strainer. If sample aggregation is a problem, we suggest using an anti-clumping buffer such as EDTA or Accumax prior to fixation.

**6. Brightness of Stain and Stain Balancing:** The sensitivity of the ImageStream is comparable to a flow cytometer. However, quantifying the location and distribution of signals in an image is a more demanding task than the measurement of simple signal strength. Therefore, follow these guidelines for the highest possible data quality:

- Adjust your staining protocols to achieve at least a full log shift over background, as measured on a standard flow cytometer.
- Use the brightest fluorochrome (ie AlexaFluor 488 or PE) for the antigen with the smallest copy number.
- The sensitivity of the instrument to different fluorochrome probes can be independently controlled. However, data quality is significantly better when the reagents used in an experiment that are excited with the same laser are titrated such that the brightness levels of all probes are balanced to within a log of each other. Probe balancing avoids the saturation of bright stains when they are combined with dim stains in the same sample.

**7. Fixation:** If fixation is desired, thoroughly fix cells (i.e. 1% PFA on ice for 20 minutes).

**8. Final Sample Concentration and Volume:** At least 1 million cells in 50  $\mu\text{L}$  ( $2 \times 10^7$  cells/ml) of protein containing buffer (ie PBS/2%FBS) in a 1.5mL siliconized microcentrifuge tube (Sigma T4816).

**9. Number of samples:** No more than 20 total. Please limit the samples to the following:

- Positive biologic control
- Negative biologic control
- Experimental samples
- Single color and unlabeled controls in separate tubes

## 10. Documentation:

- A sample list with detailed description(s) of the sample preparation protocols
- Final measured cell concentrations
- IMPORTANT: To verify sample quality upon receipt, we rely on microscope images and flow cytometry data you've acquired from the samples before sending them to Amnis. Please include these data in the shipment.

## 11. Shipping recommendations:

- Samples must be fixed and non-pathogenic.
- Wrap sample tubes in Parafilm.
- Insulate by placing in 15ml conical rack with Styrofoam lid.
- Pack in 3" thick Styrofoam box with refrigerant packs and peanuts or paper. For cold weather delivery (winter), use room temperature packs to prevent freezing of sample. For warm weather delivery (summer), use frozen packs to keep sample cool.
- Place the Styrofoam box in a corrugated cardboard box.
- Label outside of box 'Do Not Freeze'.
- Include ALL of the above documentation.
- Email the package tracking number to your Amnis contact person.

## 12. International Shipments

To ship non-viable (fixed) material internationally to Amnis, a written statement for US Customs and Border Protection, Department of Homeland Security **must** accompany the shipment. ***This written statement must:***

- be addressed to US Customs and Border Protection, Department of Homeland Security.
- be an original copy on institutional letterhead signed by the laboratory worker responsible for preparing the samples.
- identify the material and name of the species from which the material was derived.
- state that the animals from which the material was derived:
  - a. have not been exposed to, or inoculated with, any livestock or poultry disease agents exotic to the United States, and
  - b. did not originate from a facility where work with exotic disease agents affecting livestock or avian species is conducted
- state that the material is non-viable
- be placed in an envelope addressed to 'US Customs and Border Protection, Department of Homeland Security' and attached to the **outside** of the shipping box where it will be readily available for review by the USDA Inspector at the port of arrival.

If you have questions about any of the above points, please don't hesitate to contact us.

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# ImageStreamX Fluorochrome Chart

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Excitation Laser (nm)												
Ch	Band (nm)	405	488	561	592	642	785	Target	Fluor	Ch		
1	430-480	<p><b>BRIGHTFIELD</b></p> <p><b>FITC, AF488, GFP, YFP,</b> DyLight488, PKH67, Syto13, SpectrumGreen, LysoTrackerGreen, MitoTrackerGreen, QD525*</p> <p><b>PE, PKH26, Cy3, AF555,</b> DSRed</p> <p><b>PE, Cy3, AF546, AF555,</b> DyLight649, DyLight594*, PKH26, DSRed, SpectrumOrange, MitoTrackerOrange</p> <p><b>PE-TeXRed*, ECD* PE,</b> AF610*, 7AAD*, PI*, RFP, QD625*, eFluor625*</p> <p><b>PE-Cy5*, PE-AF647*,</b> PerCP*, PerCP-Cy5.5*, DRAQ5*, QD705*, eFluor650*</p> <p><b>PE-Cy7*, PE-AF750*,</b> QD800</p>										1
2	480-560											1
3	560-595											1
4	595-640											1
5	640-745											1
6	745-800											1
7	430-505	<p><b>DAPI, Hoechst, PacBlue,</b> CascadeBlue, AF405, eFluor405, DyLight405, CFP, LIVE/DEAD Violet</p>										7
8	505-570	<p>PacOrange, CascadeYellow, AF430, QD525*</p>										8
9	570-595	<p><b>BRIGHTFIELD</b></p> <p><b>TexRed*, AF594*, AF568*,</b> AF610*, DyLight594*, PE, TexRed*, ECD*, PE-AF610*, mCherry*, SpectrumRed, PI*, 7AAD*</p> <p><b>APC, AF647, AF660, AF680, AF680, APC, AF647, AF660,</b> Cy5, DyLight649, PE-AF647*, PE-<b>AF680, DRAQ5</b>, Cy5, DyLight649, DyLight680, PE, AF647, PE-Cy5, PerCP, PerCP- Cy5.5</p> <p><b>APC-Cy7, APC-AF750,</b> APC-eFluor750, Cy7, AF750, DyLight750, eFluor750, PE-Cy7*, PE-AF750*</p>										9
10	595-640											9
11	640-745											9
12	745-800											9

Dyes with an \* are excited by at least one laser directed to camera1 and another directed to camera2. The channel that the dye will appear brightest in depends on the relative laser powers used. Recommended dyes (based on optimal excitation and detection channels) are in boldface. QD565 and QD585 are not included because their primary fluorescence appears in the Ch9 Brightfield reference image.

