

SOP-E009

Standardizing the MoFlo XDP for Cervical Cancer Sorts

Objective: To standardize the XDP for Cervical Cancer Samples.

Procedure:

1. Run the standard alignment beads (SpectrAlign-Spherotech Ultra Rainbow 3.0um Beads). Verify the instrument alignment. Record the mean channel numbers on each PMT, as well as the High Voltage (HV) and Gain (G) settings. The protocol is in the following path: D:\QC**SpectrAlign_488_UV_647_20110913.plo**
2. Run the PE Reference Standard Bead in order to standardize the XDP for the PE labeled antibody (P16) on fluorescence channel two. The protocol is located: D:\Cervical Cancer\protocols**PE_Cy5experiment_20111215.plo**

Bangs R- Phycoerythrin (PE) Reference Standard Bead
Cat# 899
7.56um
Excitation: 488nm / emission: 575nm

The target mean value for the PE beads is 380. The peak for the PE reference beads should be within a couple of channels of this. If it is not, then change the HV until the mean of the peak is within a few channels of 380. Record the actual mean, and the HV setting to get it there, on the Reference Bead Log sheet.

3. Run the Cy5 Reference Standard Bead in order to standardize the fluorescence on the Cy5 channel which is on fluorescence channel six.

Bangs Cy5 Reference Standard Bead
Cat# 895
7.64um
Excitation: 649nm / emission: 666nm

The target mean value for the Cy5 beads is 3898. The peak for the Cy5 beads should be within a couple of channels of 3898. If it is not, then adjust the high voltage until it is. Record the actual mean and the HV on the Reference Bead Log sheet.

4. When the instrument alignment and the fluorescence standardization have been completed, proceed to running the samples. Follow Andy's format for naming conventions. Year(XXXXXX) month day cell line stain ex: 120123 HeLa p16 MCM5
5. Use protocol: D:\Cervical Cancer\protocols**PE_Cy5experiment_20111215.plo**
6. Save the bead files

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Date: 01-30-12

Verified by: _____ Date: _____
Print Name Sign Name