EYRAplex flow cytometer setup

EYRAplex assays are designed for measuring concentrations of multiple soluble analytes in a single sample. The assay utilizes magnetic beads labeled with APC and APC-Cy7 dye equivalents* for bead identification, and PE for analyte detection. This document will guide you through the technical requirements and recommendations for setting up EYRAplex data acquisition on most flow cytometers.

*Please note that for ease of reading, we will refer to the dye equivalents as APC and APC-Cy7

Instrument requirements:

- Flow cytometer equipped with:
- 488 nm (blue) laser for PE excitation*
- 635 nm (red) laser for APC and APC-Cy7 excitation
- Pasteur pipettes (sterile; plastic or glass)

Appropriate filters:

- PE: 575/25 nm
- APC: 660/20 nm
- APC-Cy7: 780/60 nm

* 532 nm (green) and *561 nm (yellow-green) may also be suitable for PE excitation, but EYRAplex performance has not been evaluated by Mabtech using these lasers

Instrument setup procedure:

1. Start the flow cytometer according to the manufacturer's instructions and internal standard operating procedures, including any washing, priming, and warm-up times.
2. Open a new experiment/protocol.
3. Create a dot plot with log scales for Forward scatter (FSC) vs Side scatter (SSC).
4. Add 20 µl of the unused EYRAplex Capture beads mix that were leftover following the EYRAplex assay to a FACS tube with 80-150 PBS and vortex.
5. Begin acquiring beads on set-up mode at a low flow rate (~15 mL/min.).
6. Adjust the FSC and SSC voltage/gain until the bead population can be visualized in the dot plot. The flow rate may be adjusted if needed.
7. Make a "Beads" gate and drill through to make a new dot plot with SSC-H and SSC-A with log scales.
8. Make a "Singlets" gate along the diagonal and drill through to create a new dot plot with APC (660 nm) vs APC-Cy7 (785 nm) using log scale.
9. Adjust the detector voltage/gain for both the APC and APC-Cy7 channels until each of bead populations is on scale. The number of populations will be determined by the plex of the EYRAplex kit.
10. Once all distinct bead populations are visible, stop acquisition and make gates around each bead population, labeling with the analyte as shown in the population map of the EYRAplex assay datasheet.
11. In order to set the PE voltage, open a PE histogram from the "Singlets" gate or open one histogram for each analyte gate. Display "median fluorescence intensity (MFI)" and "count" statistics for each plot.
12. Adjust the PE detector voltage/gain such that the PE signal is in the first decade. At this point, you may want to spot check a stained EYRAplex sample that you anticipate to have the highest PE signal (e.g. neat standard) to make sure the peak is not off-scale positive. Refine the PE detector voltage/gains using the unstained bead sample and the highest analyte concentration sample such that both are on scale.
13. Save the instrument settings, disable set-up mode, and acquire samples, collecting a minimum of 400 bead events per individual analyte

No compensation is required; however a user may optionally acquire separately prepared compensation samples (such as comp beads singly stained with PE, APC, or APC-Cy7 antibodies, not included in EYRAplex kits) at the same settings and apply the resulting matrix.

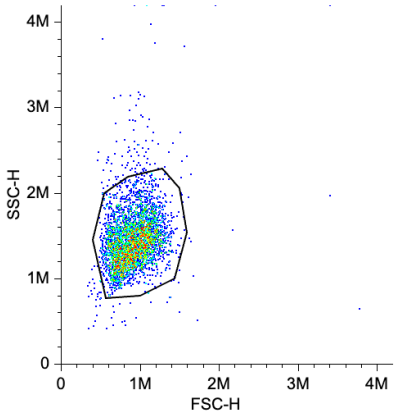
Tip
Always keep an eye on the beads FACS tube to be sure you have enough volume for the setup procedure.

Data acquisition procedure:

1. Adjust acquisition settings based on whether you are using FACS tubes or plates with flow rates designated by internal SOPs.
2. Acquisition settings should be set to a minimum of 400 beads per bead population gate.
3. Acquire and save the data acquisition files for data analysis.
   a. If your instrument allows, you may also export the PE MFI values from each bead population directly into an Excel file. Be sure that bead populations and samples are correctly labeled before exporting. The next section can then be skipped.

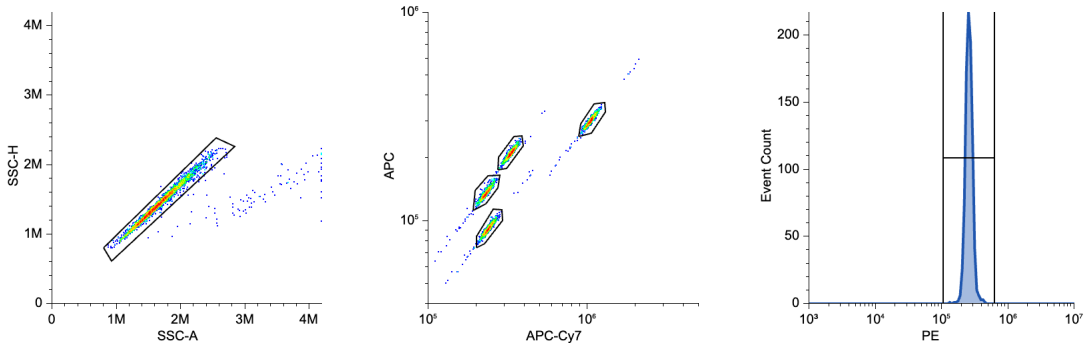
General data analysis procedure:

1. Load the data acquisition files into your FACS analysis software of choice (e.g., FlowJo).
2. Open a new protocol or experiment and import the data files.
3. Starting with the highest standard dilution well, create a dot plot with FSC and SSC to select the bead populations from debris



4. Continue the gating strategy as described previously with a singlet's gate, APC/APC-Cy7 gate, and PE-histogram plot.

   a. Place gates around every bead population and label with the correct analyte as noted in the EYRAplex assay datasheet's population map.



5. Depending on the analysis software, you may create a statistics table displaying the PE median fluorescent intensity (MFI) from each bead gate.
   a. You may also first have to drill through and create a histogram plot and gate the positive PE signal for each bead population before creating the statistical table.
6. Apply the gating strategy to the rest of the standard and sample data files.
7. Export the bead values' PE MFI values and continue analysis in a suitable software for five-parameter logistic (5PL)analysis, such as GraphPad Prism. Use the generated standard curves to generate the unknown sample(s) concentrations for each analyte.

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