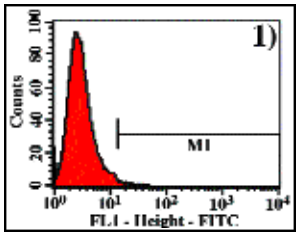
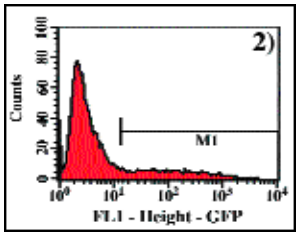
# FACS data: Positive Controls

*by Brian Russell*

The goal in most FACS experiments is to see positive expression of the antibody/dye complex, indicating specific binding to the cell or receptors in question. The combination of an antibody that binds specifically to the cell and a fluorescent dye that binds or is conjugated to the antibody, will result in the histogram peak moving to the right in some manner or form as compared to the control peak. There are a variety of shapes to the curves of histograms that all demonstrate positive expression.

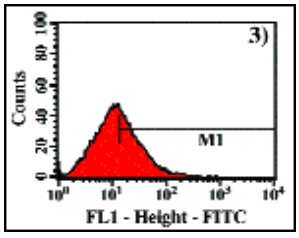



1. Remember from last issue, the control peak is what is used to establish the baseline fluorescence intensity by adjusting the unstained, or singly stained for background fluorescence, cells peak to the left of the histogram's X-axis as shown in the first figure. A marker (M1) is then set with <1% of the control peak inside the marker's left edge, this being the standard for comparison when it comes to the positively-expressing samples. Examples 2-5 show positive histogram peaks.

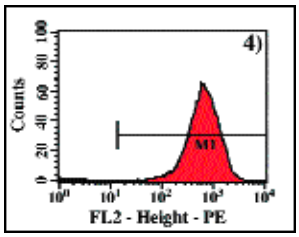



2. The gradual down-slope of this curve indicates that there is a broad range of fluorescence intensity, which correlates to a wide range in the number of molecules of the dye/antibody complex that bind to the cells. As the fluorescence intensity increases along the X-axis, there seem to be fewer and fewer cells at that intensity resulting in a very broad, yet short (in cell number), peak. A wide shoulder such as this shows there are clearly some cells that are very brightly labeled, while others demonstrate very low levels of fluorescence. One point to note: although there appears to only be a 18.74% shift, the average (mean) intensity is high at **293.89**.

| Marker | Left, Right | Events | % Gated | % Total | Mean |
|---|---|---|---|---|---|
| All | 1, 9910 | 9952 | 100.00 | 100.00 | 57.72 |
| M1 | 13, 9910 | 1865 | 18.74 | 18.74 | 293.89 |

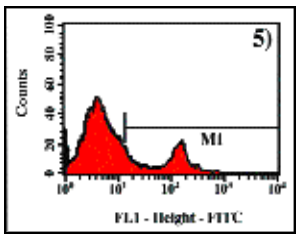



3. This sample shows a broadening of the peak as well. The edge of the peak still touches the origin, meaning not all of the cells bound the antibody/dye complex, but there are several cells that did bind, shifting the intensity to the right beyond the marker. An important observation is that the peak shifted a healthy 42%, but the mean fluorescence is still very low (39.9) compared to the previous histogram.




4. This histogram shows a sample that is very bright. Practically the entire peak has shifted into the marker region. This sample has bound very well with the antibody/dye complex, and as a result has a 99% shift. The sharpness of the peak also indicates that the cell surface may contain more antigens for the tested antibody than are shown in the previous histogram. What is even more illustrative is the "mean" at **693.03**, indicating that not only is there a 99.44% shift but the peak has shifted way beyond the edge of the marker.

| Marker | Left, Right | Events | % Gated | % Total | Mean |
|---|---|---|---|---|---|
| All | 1, 9910 | 10000 | 100.00 | 100.00 | 689.16 |
| M1 | 13, 9910 | 9944 | 99.44 | 99.44 | 693.03 |




5. There is a double peak in this bi-modal histogram. The first peak is still to the left of the marker and indicates that very few cells have bound the antibody/dye complex. There is slight shifting that could be due to non-specific binding. There is also another population that results in the second peak beyond the marker region. This second peak is indicative of some cells binding well, while others not at all. This could mean that there is a different characteristic in some of the cells of that type that causes a small percentage to bind significant fluorophore, or that there is some limit to the percentage of cells that can bind in any one sample.

**Next Issue: Dot Plots**

***You can always get something positive out of FACS!***

Understanding FACS