

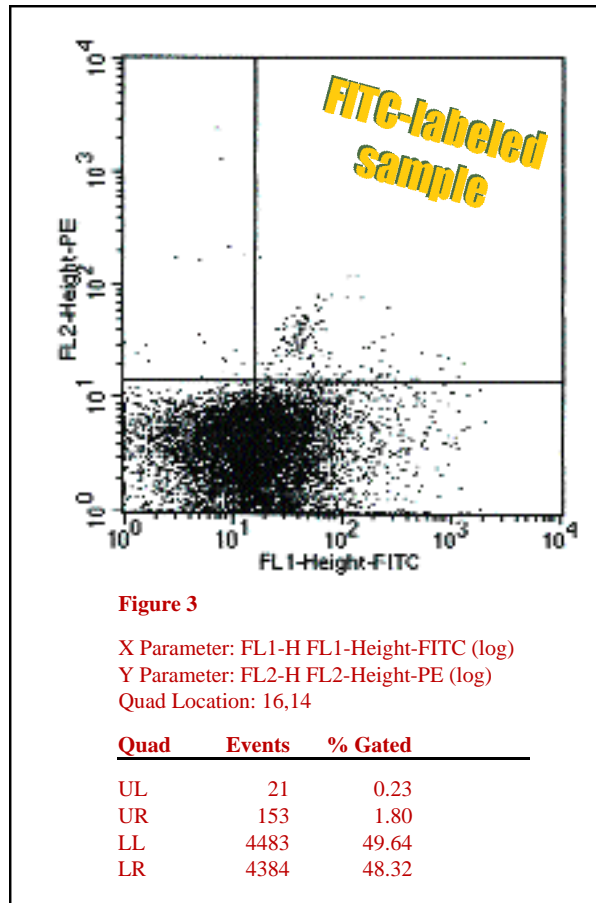
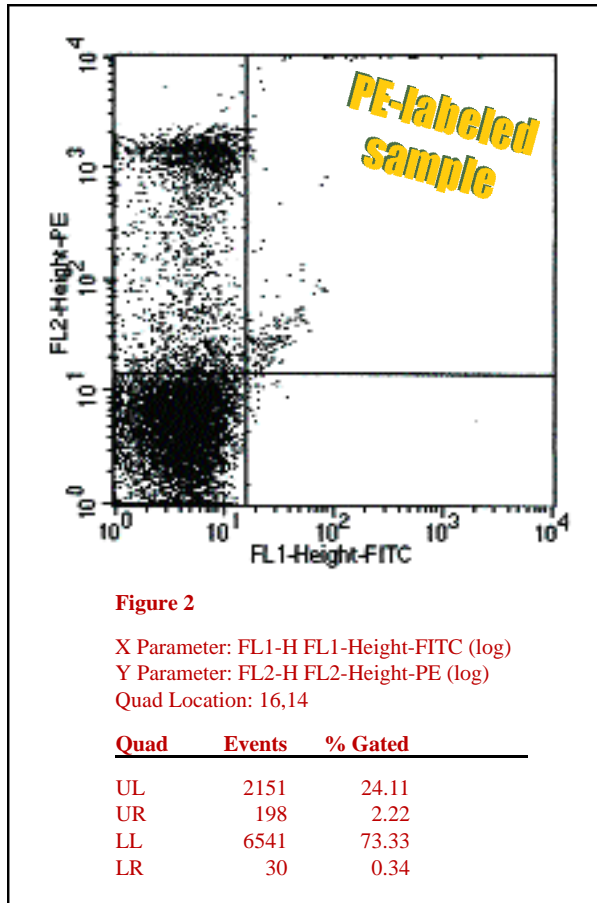
## FACS data: Multiple Color Controls

*(continued from page 2)*

The standard set of control samples for a two-color experiment is as follows. The first sample ideally should be a cells alone sample that will allow for the subtraction of the basal level fluorescence of the cells from the positives (Figure 1). There should also be a singly labeled positive sample for each of the fluorochromes (Figure 2 for PE, Figure 3 for FITC) used so that compensation can be set for each of the fluorescent parameters. By setting the voltage levels and the compensation on each of the dyes individually, it provides the proper instrument settings for the double-stained positive samples to follow. (Examples of double-stained samples will

be discussed in our next issue.) When buying pre-conjugated fluorescent antibodies most of them come with an isotype control. This control dye is what should be used to make the singly-labeled control samples for setting the voltage and compensation levels. An isotype control is a fluorochrome conjugated to non-specific Ig's (IgG, IgM, etc.) against the species of the cell type used in the experiment. In addition to helping the set-up procedure by providing compensation assistance, isotype controls also allow for the subtraction of the fluorescence associated with non-specific binding.

**You will only be certain of how good your results really are if you use the proper controls!**



**Next Issue:  
Two-color Assays**