Bangs Laboratories, Inc.
BEADS ABOVE THE REST

Determination of Effective F/P Ratio

Product Data Sheet 849

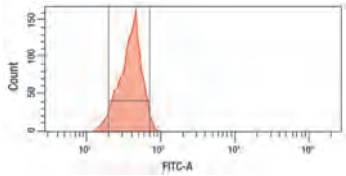
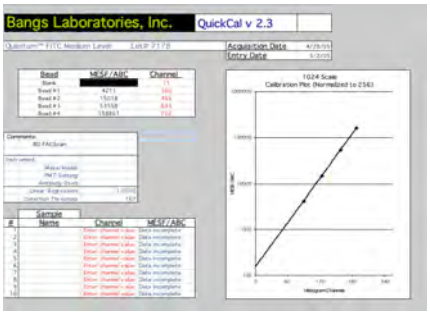
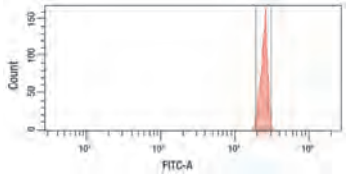
In flow cytometry, the **effective F/P ratio** is the average apparent number of fluorochrome molecules conjugated per primary antibody, as determined through fluorescence measurements taken on the flow cytometer. Specifically, beads that are calibrated in terms of ABC (Antibody Binding Capacity, or the # of antibodies able to be bound per bead), are stained with the fluorophore-labeled primary antibody. The labeled beads are run on the cytometer against a curve generated with MESF beads (Molecules of Equivalent Soluble Fluorochrome, a standardized measurement of fluorescence intensity) to also assign an MESF value. In this way, the fluorescence intensity of a known # of antibodies (and, by extension, per antibody) may be determined.

STAINING AND RUNNING MESF & SC BEADS

1. Run the appropriate MESF beads on the flow cytometer and obtain the median channel fluorescence values for each peak (see PDS 821 for more info).
2. Prepare and stain the Simply Cellular beads with the relevant flurochrome-conjugated primary antibody as directed in PDS 810.
3. Run the stained Simply Cellular beads on the flow cytometer at the same settings used for the MESF beads and obtain the median channel fluorescence value.

INPUTTING DATA AND CALCULATING EFEECTIVE F/P RATIO

1. Enter MESF beads' channel values into the appropriate QuickCal template (see PDS 821 and 819) to form the standard curve.
2. Enter the Simply Cellular bead channel value into sample cell 1.
3. Take the derived MESF value for the Simply Cellular bead and divide by the ABC listed on the Simply Cellular CoA. The result is the effective F/P ratio.
4. The F/P ratio may be used to convert sample MESF values into quantitative antibody binding, as long as the same antibody lot and MESF kit is used. For example, a cell sample was measured with a MESF value of 320,000. The F/P of the antibody was previously determined to be 2.5 fluorochromes per antibody. 320,000/2.5 = 128,000 antibodies bound.

Step 1	Step 2	Step 3	Step 4
Label **Simply Cellular** with FITC-Ab	Determine FI (channel value)	Enter channel values in **QuickCal®** (against curve from **Quantum FITC MESF** kit)	MESF ÷ ABC = **effective F/P**
? mAb1			9,738 ÷ 9,066 = 1.07
? mAb2			26,959 ÷ 9,066 = 2.97

Note: Antibody suppliers may provide information regarding the fluorophore labeling density of the anitbody. These are often measured via absorbance studies to determine the molar concentrations of both the flurochrome and protein. Differences between the effective F/P via fluorescence/flow cytometry and F/P (via absorbance studies) may exist. This is due to differences in the method, as well as the measured fluorescence of the fluorochrome once conjugated to the protein. The effective F/P is also specific to the antibody Lot used. If a new Lot is received, a new measurement must be taken.