QuantiBRITE™ PE*

QuantiBRITE PE

Catalog Number: 340495

Number of Tests/Kit: 10

Phycoerythrin Fluorescence Quantitation Kit

Description

Each Becton Dickinson QuantiBRITE PE tube contains a lyophilized pellet of beads conjugated with four levels of phycoerythrin (PE). The pellet is restrained in the bottom of the tube by a stainless steel retainer. The number of PE molecules per bead at each level varies among lots; lot-specific information is included with each kit.

Research Application

Flow cytometric estimation of antibodies bound per cell (ABC)[1,2]

Principles of Procedure

QuantiBRITE PE tubes are designed for use with PE-labeled monoclonal antibodies for the purpose of estimating ABCs by flow cytometry. When you run a QuantiBRITE PE tube at the same instrument settings as the assay, the FL2 axis can be converted into the number of PE molecules bound per cell. By using known ratios of PE to antibodies, you can then convert PE molecules per cell to antibodies per cell. These factors, and others that affect quantitation, are discussed in detail in the QuantiBRITE white paper.[3]

Method

Preparation

Remove the QuantiBRITE PE tube from the foil pouch just prior to use, reconstitute using 0.5 mL of buffer, such as PBS with azide plus 0.5% BSA, and vortex.

Flow Cytometric Analysis

- Launch CellQuest™. Using the setup mode, adjust all parameters for your cellular assay. Make sure the instrument is compensated properly, for example, with CaliBRITE™ beads.
- Open the Quantitation Acquisition document located in the Sample Files folder in the CellQuest folder (only in CellQuest 3.1 and later versions). Modify the document to include plots for your cellular assay.
- Run the QuantiBRITE PE tube, thresholding on FSC or SSC, and collect 10,000 events. The FSC and SSC parameter settings can be changed to gate on bead singlets without altering quantitation (Figure 1). All instrument settings for fluorescence and compensation must be the same as the cellular assay settings.

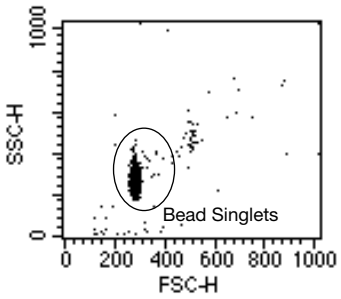

Figure 1

- Adjust the gate around the bead singlets (Figure 1) on the FSC-H vs SSC-H plot. The singlet bead population is analyzed using a histogram plot of FL2-H in linear values.
- Adjust markers around the four bead peaks (Figure 2). View the histogram statistics (Figure 3), making sure that the geometric means are displayed.

* US Patent No. 4,520,110; European Patent No. 76,695; and Canadian Patent No. 1,179,942.

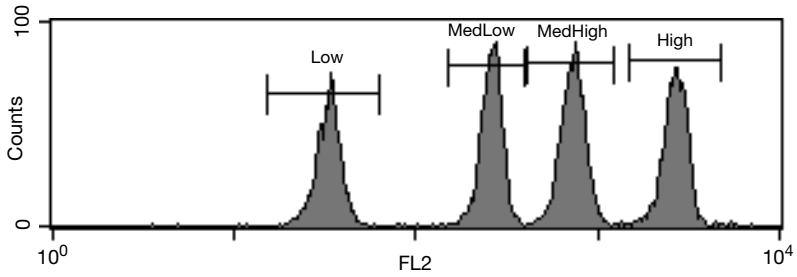

Figure 2

Histogram Statistics

File: SI06066.012 — Log Data Units: Linear Values
Sample ID: — Acquisition Date: 6-Jun-96
Gate: Bead Singlets — Gated Events: 9919
Total Events: 10000 — X Parameter: FL2-H (Log)

Marker	Left, Right	Events	Geo Mean	CV
All	1, 9910	9919	393.20	109.16
Low	15, 62	2044	32.28	14.26
MedLow	149, 392	2540	250.48	12.05
MedHigh	407, 1219	2817	699.69	14.29
High	1472, 4698	2494	2530.73	13.30

Figure 3

- Select the Histogram Statistics view and choose Quantitative Calibration from the Acquire menu.
- Click the Copy Means button to copy the geometric means of the four bead peaks from the histogram statistics window.
- Enter the lot-specific PE/bead values provided on the flyer packaged in the QuantiBRITE PE kit.
- Press the tab key; then click Calibrate for CELLQuest to perform the regression analysis, and to display the slope, intercept, and correlation coefficient.
- Save the Experiment document.
- Using the same instrument settings and Experiment document, acquire your cellular assay samples. All subsequently collected data files will save the information displayed in the Quantitative Calibration window. You can print an active Quantitative Calibration window by selecting Print from the File menu. QuantiCALC™ can read the regression information for analysis of assay files.

For detailed information on using the Quantitative Calibration option, refer to Chapter 10 of the *CELLQuest Software Reference Manual* (version 3.1 or later).

Manual Analysis

Use the following procedure to calculate the PE molecules per cell of the population of interest if you do not have CELLQuest 3.1 or later revisions.

1. On a statistics spreadsheet, enter the geometric means from the Histogram Statistics view (Figure 3) for the four beads.
2. Enter the lot-specific values for the PE molecules per bead (provided in each QuantiBRITE PE kit box).
3. Calculate the Log_{10} for the FL2 geometric means and for the PE molecules per bead as illustrated in the following table.

   Example:

FL2 Geometric Means	Log FL2	PE Molecules/Bead	Log PE Molecules/Bead
32.28	1.508	1700	3.230
250.48	2.399	14200	4.152
699.69	2.844	39400	4.595
2530.73	3.403	133400	5.125

4. Plot a linear regression of Log_{10} PE molecules per bead against Log_{10} fluorescence, using the following equation:

$$y = mx + c$$

where *y* equals Log_{10} fluorescence and *x* equals Log_{10} PE molecules per bead (Figure 4).

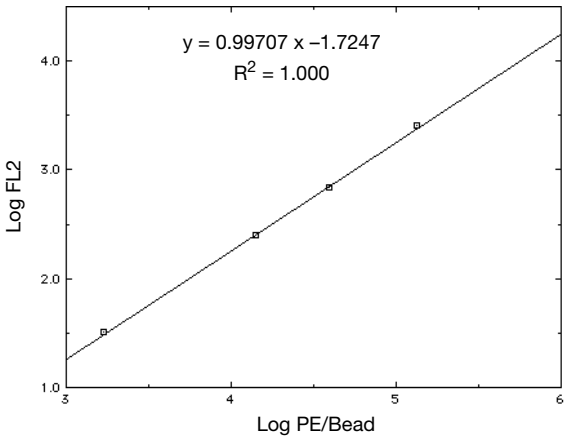

Figure 4

5. To determine ABC* for an unknown cell population, substitute Log FL2 geometric means in the equation and solve for Log ABC. Determine the anti-Log to get ABC.

Example:

If FL2 fluorescence of the cell population is 500, and Log_{10} 500 = 2.699, using the equation from Figure 4, y = 0.99707x – 1.7247, we solve for *x*:

$$2.699 = 0.99707x - 1.7247$$

$$x = \frac{2.699 + 1.7247}{0.99707} = 4.4367$$

$$x = Log_{10} \text{ PE/cell} = 4.4367$$

$$\text{PE molecules/cell} = 27{,}334$$

When PE:mAb ratio is 1:1, then: ABC = 27,334

* This calculation assumes a PE to mAb ratio of 1:1.

HANDLING AND STORAGE

Each QuantiBRITE PE tube contains one lyophilized bead pellet. Each tube is packaged in a foil pouch. Store foil pouches at 2° to 8°C and reconstitute the pellet immediately after removal from the pouch.

The reconstituted pellet is stable for 24 hours when protected from light and stored at 2° to 8°C.

LIMITATIONS

Factors that can affect quantitation include, but are not limited to, fixation, source of antibody, and clonal variation. Refer to the QuantiBRITE white paper for more information.[3]

WARRANTY

The products sold hereunder are warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, which extend beyond the description on the label of the product. Becton Dickinson's sole liability is limited to either replacement of the products or refund of the purchase price. Becton Dickinson is not liable for property damage, personal injury, or economic loss caused by the product.

REFERENCES

1. Davis K, Abrams B, Hoffman RA, Bishop JE. Quantitation and valence of antibodies bound to cells. *Cytometry.* 1996; #AC150 (Suppl. 8):150.

2. Iyer S, Suni M, Davis K, Maino V. Expression of CD69 on activated T cells using R-phycoerythrin labeled beads. *Cytometry.* 1996; #AC78 (Suppl.8):113.

3. Iyer S, Bishop J, Abrams, B, et al. *QuantiBRITE: A New Standard for Fluorescence Quantitation.* Becton Dickinson Immunocytometry Systems, San Jose, CA. 1997. White Paper.

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