

9025 Technology Dr. • Fishers, IN 46038-2886
 800.387.0672 • 317.570.7020 • Fax 317.570.7034
 info@bangslabs.com • www.bangslabs.com



B E A D S • A B O V E T H E R E S T™

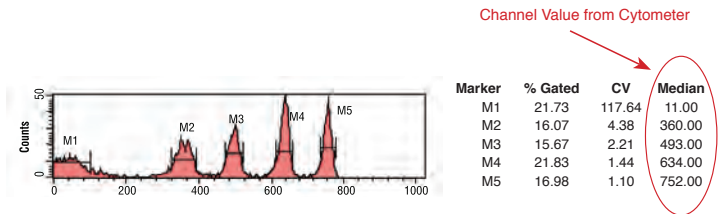
DESCRIPTION

QuickCal® v. 2.3 is an Excel® spreadsheet-based analysis template that is intended for use with Quantum™ MESF and Quantum™ Simply Cellular® kits. Specifically, QuickCal® simplifies quantitative fluorescence analysis through pre-programmed regression calculations. The resulting calibration curve associates fluorescence channel values to standardized fluorescence intensity units (MESF or ABC), and permits the assignment of MESF or ABC values to labeled cell samples. QuickCal® also serves a QC function, reporting both the lower detection threshold and regression coefficient (linearity) for the pertinent detector at test-specific settings.

Note: ABC - Antibody Binding Capacity; Quantum™ Simply Cellular® products
 MESF - Molecules of Equivalent Soluble Fluorochrome;
 Quantum™ MESF products

PROCEDURE

- Go to the Bangs Laboratories' website to download the QuickCal® template at www.bangslabs.com/quickcal.
- Enter relevant information, including your QuickCal® Access Number, at the prompt.
- Select the file you would like to download. *Note:* Files are lot-specific.
- Select the appropriate version based on the resolution of your instrument. Options include:
 - 256 channels for linear data
 - 1024 channels for linear data
 - 4096 channels for linear data
 - Logarithmic regression (e.g. BD FACSDiva™, Accuri™ C6) for linear data
 - BD Relative Linear Scale for log data
 - Coulter Relative Linear Scale for log data
- If using Quantum™ FITC-5 MESF, select the intensity range you need. Options include:
 - Low four intensity template
 - High four intensity template
 - All five intensity template
- The selected QuickCal® template will download to your computer.
- Run Quantum™ MESF or Quantum™ Simply Cellular® microspheres on the same day and at the same instrument (PMT and compensation) settings as the labeled cell samples. Document relevant details (PMT voltage, mAb clone, etc.) in the "Instrument" and "Comments" sections of the template
- Gate on each population within the fluorescence histogram.
- Enter the Median or Geo Mean channel value for each fluorescence peak against its calibrated MESF or ABC value that appears within the QuickCal® (Excel®) template in the "channel" field in the first section. Both the regression coefficient and detection threshold will be reported.
- Enter the Median or Geo Mean channel values of labeled samples for the assignment of MESF or ABC values.



Bangs Laboratories, Inc. QuickCal v 2.3

Quantum™ MESF Lot# xxxxx Acquisition Date dd/mm/yy
 Entry Date dd/mm/yy

Bead	MESF/ABC	Channel
Blank	11	257310
Bead #1	4211	360
Bead #2	15018	496
Bead #3	33558	634
Bead #4	158861	752

Comments:

Instrument: Make/Model: PMT Setting: Antibody Used: Linear Regression: 1.0000 Detection Threshold: 1.67

#	Sample Name	Channel	MESF/ABC
1	Sample 1	568	40,139
2	Sample 2	603	40,139
3	Sample 3	568	29,051
4		Enter channel value	Data incomplete
5		Enter channel value	Data incomplete
6		Enter channel value	Data incomplete
7		Enter channel value	Data incomplete
8		Enter channel value	Data incomplete
9		Enter channel value	Data incomplete
10		Enter channel value	Data incomplete

1024 Scale Calibration Plot (Normalized to 256)

Channel Value from Cytometer →

MESF (or ABC) assignment of stained cell samples →

NOTES

- The QuickCal® Access Number is printed on a label affixed to the product canister. In most cases, this is the same as the invoice number for the specific order. (See sample label below.)
- Use Bangs Laboratories' new online quantitative software, QuickCal v 2.3 to analyze your flow cytometry data. To access QuickCal v 2.3, go to www.bangslabs.com/quickcal and enter access code: [CODE]

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- Generally, the Median or Geo Mean channel value is entered for each bead population. Either statistic may be used, though the same must be used for beads and cell samples.
 - The calibration (regression) curve is calculated from values entered for the four (or five for FITC-5) labeled bead populations. The blank bead population is not used to construct the curve, but is read from the curve, and reported as the detection threshold. The regression coefficient (r²) is also reported. R² values as near as possible to 1.0 are desired. Sub-optimal linearity will impact the accuracy and reproducibility of assignments to cells and may indicate: a) a problem with the run; b) use of the wrong template resolution; or c) the need for instrument maintenance.

TROUBLESHOOTING

- If the curve doesn't fit the window, it's likely that the wrong version of the template has been used. To determine "resolution," or the appropriate version of the template, look at the x-axis of the

fluorescence histogram. Typically, numbering of 0 - 1000 = 1024 template; $10^0 - 10^4$ = BD Relative Linear; 0.1 - 1000 = Coulter Relative Linear.

2. An unexpectedly high detection threshold may indicate free dye in the system, or, for Quantum™ Simply Cellular®, that the blank bead population was stained with the antibody-coated beads.
3. If poor results (e.g. linearity) are achieved with a particular run, prepare and run a fresh sample. If poor results persist, this may indicate:
 - (for Quantum™ Simply Cellular® beads) a problem with staining;
 - the bead sample was damaged, e.g. photobleached;
 - the need for instrument service.

TRADEMARKS AND REGISTERED TRADEMARKS

1. Quantum™, QuickCal®, and Simply Cellular® are trademarks or registered trademarks of Bangs Laboratories, Inc.
2. Accuri™ and FACSDiva™ are trademarks of Becton Dickson and Company.
3. Excel® is a registered trademark of Microsoft Corporation.
4. Alexa Fluor® is a registered trademark of Life Technologies Corporation.
5. Cy™, including Cy5, are trademarks of GE Healthcare Limited. These products are manufactured under license from Carnegie Mellon University under U.S. Patent Number 5,268,486 and related patents.

These products are for research use only and are not intended for use in humans or for *in vitro* diagnostic use.

ORDERING INFORMATION

Cat. Code	Description	Sizes
488	Quantum™ Alexa Fluor® 488 MESF	1mL, 5mL, or 14mL
647	Quantum™ Alexa Fluor® 647 MESF	1mL, 5mL, or 14mL
823	Quantum™ APC MESF	1mL, 5mL, or 14mL
822	Quantum™ Cy™5 MESF	1mL, 5mL, or 14mL
555	Quantum™ FITC-5 MESF	1mL, 5mL, or 14mL
555p	Quantum™ FITC-5 MESF Premix	1mL, 5mL, or 14mL
827	Quantum™ R-PE MESF	1mL, 5mL, or 14mL
828	Quantum™ PE-Cy™5 MESF	1mL, 5mL, or 14mL
815	Quantum™ Simply Cellular® anti-Mouse IgG	1mL, 5mL, or 14mL
816	Quantum™ Simply Cellular® anti-Human IgG	1mL, 5mL, or 14mL
817	Quantum™ Simply Cellular® anti-Rat IgG	1mL, 5mL, or 14mL

Order online anytime at www.bangslabs.com.