

DESCRIPTION

Quantum™ products are somewhat specialized, and presume a basic proficiency in sample preparation and handling, as well as cytometer operation. This document is intended to supplement the Product Data Sheets by providing tips for working with the Quantum™ products. Problems are often the result of sub-optimal conditions or basic errors, and there are often simple strategies to improve results.

Notes: QSC beads are coated with capture antibodies, and require staining by the user; MESF beads are pre-labeled and do not require staining. Please note that some of the comments below are specific to bead staining protocols and, by extension, to QSC kits.

MESF - Molecules of Equivalent Soluble Fluorochrome

ABC - Antibody Binding Capacity

Handling Considerations

- The fluorescence intensity of fluorochromes (e.g. FITC) can be highly dependent upon the pH, ionic strength, etc. of the suspending solution. For that reason, the beads must be suspended in the same type of buffer or medium as the stained cells when runs are performed.
- Remove the bottles from and return them to the refrigerator as quickly as possible (<5 minutes) to avoid the cumulative negative effects of repeated temperature cycling.
- Do not vortex or sonicate stock bottles. Prepared samples may be vortexed briefly in the tube to increase % singlets if needed, vigorous tapping/flicking also helps.
- Prepare all suspensions immediately prior to use.
- Bead populations may be stained/run individually (standard kits) or as mixed populations. If having resolution issues, running the populations individually will ensure best gating.

Getting Started

- Get to know your instrument. For accurate MESF or ABC assignments, instrument linearity must be assured. A regression coefficient ≥ 0.9995 is generally desired.
- Conduct an antibody titration for the QSC beads so you are confident that saturation is being achieved (the antibody concentration used for cells may not be optimal for the beads). Typically bead 4 is titrated and the resulting value is assumed to be sufficient for the lower beads. See PDS 814A.
- Ensure that the labeled cells and beads appear in the same window of analysis (i.e. are on scale) when run at the same fluorescence (PMT and compensation) settings.
- The standards must be analyzed on the same day, on the same instrument, and at the same fluorescence (PMT and compensation) settings as stained cell samples, although forward and side scatter settings (FSC, SSC) may be adjusted to optimize dot plot gating.
- Use the same lot of the same Ab clone for the duration of the study. Where a new lot must be used, run QSC bead samples stained with each lot in parallel to identify any variation in staining.
- Using a fluorescent bead standard with each run can help in identify one-off sample preparation problems, etc. For example, using a hard-dyed bead such as Quantum™ QC provides a reference point for each run.

TROUBLESHOOTING AND COMMON PROBLEMS

No or Poor Fluorescence

- Ensure that the FSC/SSC gate has been applied to the fluorescence histogram (double-check histogram labels; the template may have been inadvertently changed).
- Confirm that the primary mAb host species is suitable for the QSC kit. The host species of the antibody is key, not the target cell population. For example, a mouse anti-human antibody would require our QSC anti-mouse IgG kit. Whole IgGs with an intact Fc region are required - the beads bind the Fc region.
- Protect the fluorochrome-conjugated Ab, stained samples, and beads from light to prevent photobleaching.
- Ensure that the laser and detector are suitable for the reporter fluorochrome.
- In the special case of Fc-tagged proteins, they should be tested to ensure acceptable binding to the Fc-specific antibody coated on the beads. We have known some Fc tags to exhibit different binding than their native Ab counterparts, and a lack of binding in rare instances.

Broad Fluorescence Peaks or Poor Resolution

- Lower peaks may have some overlap, especially the blank and bead 1. This is not an issue as long as bead 1 has a higher channel value.
- Peak spacing can vary kit to kit, Lot to Lot. Some products may have closer proximity of peaks than others.
- Lower intensity beads will naturally have broader peaks due to the photoelectron statistical error of the instrument's PMT.
- Try higher/lower PMT values, very low or high PMTs could cause poor peak CVs.
- Ensure that only singlets are gated, any extraneous populations will increase peak CVs.
- Broad QSC peaks may indicate that saturation has not been achieved; an antibody titration will aid in ensuring that bead samples are stained to saturation. See PDS 814A.

- Do not stain the blank population (the blank should be kept in a separate tube).
- Use of an indirect staining approach will lead to broader QSC bead peaks. Populations should be stained and run separately for optimal gating.

General

- If you achieve poor results with a particular run, stain and run a new sample.
- Remove all compensation settings to evaluate whether this may be contributing to any issues.
- Staining and running peaks separately may provide more specific information for troubleshooting.
- Labeling QSC beads with a different antibody (clone and fluorophore) will aid in identifying clone or fluorochrome-specific effects.
- Low event rates or issues in resolving the bead FSC/SSC almost certainly indicates an FSC/SSC settings issue. Be sure to check the scale, settings, and thresholds. Most often this occurs when cells were previously analyzed, and the FSC/SSC settings have been reduced to get the larger cells in-scale (QSC/MESF beads are ~ 7 μm).
- High amounts of aggregates can be resolved by aggressive flicking/tapping of the bead tubes. Be sure to not over pellet the spheres using centrifugation as well.
- Contact your institution's core flow cytometry facility for access to experts in sample labeling, instrument operation, etc.

QUICKCAL®

- A QuickCal analysis template is provided with each Quantum MESF or Quantum Simply Cellular kit to facilitate assignment of MESF or ABC values to cells. See Product Data Sheet #819 for detailed instructions.
- If the curve doesn't fit in the window or the regression coefficient (r^2) is very poor, 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; 10^{-1} - 10^3 = Coulter Relative Linear, and 10^1 - 10^6 (or higher) = log/log (for linear data).
- An unexpectedly high detection threshold may indicate free dye in the system, or that the blank bead population was stained with the antibody-coated beads. Users are also welcome to substitute their own blanks (e.g. unstained cells).
- QuickCal is a locked template to prevent inadvertent modifications of the formulas or bead assignments. We can unlock templates upon request, reach out to info@bangslabs.com.

TRADEMARKS AND REGISTERED TRADEMARKS

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ORDERING INFORMATION

Cat. Number	Description	Sizes
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
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
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
725	Quantum™ QC	5mL

RELATED TECHNICAL LITERATURE

PDS 814 - Quantum Simply Cellular
 PDS 814A - QSC/SC Antibody Titration Protocol
 PDS 819 - QuickCal, v. 3.0 Data Analysis Program
 PDS 821 - Quantum MESF
 Flow Quality Control and Standardization Brochure
 Flow Cytometry Instrument Quality Assurance / Quality Control Brochure
 Quantitative Cytometry Brochure
 Flow Cytometry Guide

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