Product Data Sheet 818

Quantum™ Simply Cellular® & Quantum™ MESF Tips and Techniques

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BEADS ABOVE THE REST™

DESCRIPTION

Bangs' tools for quantitative expression analysis include Quantum™ MESF and Quantum™ Simply Cellular® (QSC) products. Quantum™ kits are comprised of microsphere populations that are used to generate a standard curve relating fluorescence intensity to standardized MESF or ABC values. The expression levels of cells may then be determined by measuring their fluorescence intensities and reading the corresponding MESF or ABC values from the standard curve using the QuickCal® analysis template provided with each kit.

Quantum™ products are somewhat specialized, and presume a basic proficiency in both sample preparation and handling, and cytometer operation. If your institution has a core flow cytometry facility, you're in luck, as you'll have ready access to experts in these areas. However, if you're on your own (and even if you're not), we wouldn't think of abandoning you! This document is intended to supplement the Product Data Sheets by providing insider tips for working with the Quantum™ products. After all, we've seen our share of troubles over the years, and would be pleased if you could learn from our mistakes. 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

PROCEDURE

Before You Begin

- 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.
- For accurate MESF or ABC assignments, instrument linearity must be assured. A regression coefficient ≥ 0.9995 is generally desired.
- 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.

General Procedure

1. Investigators are advised to optimize the use of microsphere standards in any application. For Quantum™ MESF and Simply Cellular® kits, this includes ensuring 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.
2. Prepare all suspensions immediately prior to use.
3. Protect from light to guard against photobleaching.
4. 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.
5. Bead populations may be run individually (standard kits) or as mixed populations. If the resolution of the detector is sub-optimal, running the populations individually will ensure best gating.

Getting Started

- Conduct an antibody titration for the QSC beads so you are confident that saturation is being achieved. (Bear in mind that the antibody concentration used for cells may not be optimal for the beads.)
- Stain and run each antibody-coated population (QSC Beads 1-4) separately for at least the first run to ensure satisfactory labeling and optimal resolution for 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 suitable Fluorescent Reference Standard provides a reference point for each run.
- Get to know your instrument. Quantitative fluorescence analyses won't be accurate or reproducible if there are problems with instrument linearity, resolution, etc.

TROUBLESHOOTING

No or Poor Fluorescence

- Ensure that the primary mAb species is suitable for the QSC kit. For example, the anti-Mouse kit is intended to bind mouse mAbs, not for the analysis of mouse cells.
- 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.
- Ensure that the FSC / SSC gate has been applied to the fluorescence histogram (double-check histogram labels; the template may have been changed inadvertently).
- 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

- Use of an indirect staining approach will lead to broader QSC bead peaks. Populations should be stained and run separately for optimal gating.
- 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.
- Ensure that only singlets are gated.
- Do not stain the blank population, which consists of uncoated polymer beads that will be happy to bind antibody nonspecifically.

General

- If you achieve poor results with a particular run, stain and run a new sample.
- 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.
- If there are no or low bead counts, the sample may have been over-diluted, or there may be a problem with the instrument (laser or fluidics). If the preparation of a new sample doesn't resolve the problem, try re-booting the computer and reconnecting the cytometer.
- We also encourage you to 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, 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.
- 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.

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2. Cy™, including Cy5, is a trademark 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.
3. Alexa Fluor® is a registered trademark of Life Technologies Corporation.

RELATED TECHNICAL LITERATURE

1. PDS 814 - *Quantum™ Simply Cellular®*
2. PDS 819 - *QuickCal®, v. 2.3 Data Analysis Program*
3. PDS 821 - *Quantum™ MESF*
4. BSS 007 - *Flow Quality Control and Standardization*
5. BSS 008 - *Flow Cytometry Instrument Quality Assurance / Quality Control*
6. BSS 025 - *Quantitative Cytometry*

SAFETY

These particle suspensions may contain sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Upon disposal of material, flush with a large volume of water to prevent azide accumulation. Please consult the Material Safety Data Sheet for more information.

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
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
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

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