Product Data Sheet 814A

QSC/SC Antibody Titration Protocol

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BEADS ABOVE THE REST™

INTRODUCTION

Our Quantum Simply Cellular (QSC) and Simply Cellular (SC) product lines are intended for the quantitation of antibody binding capacity (ABC) of various cell samples. Quantitation of receptor density is a highly demanding application, and accurate results require that each bead's binding sites are fully saturated with antibody (otherwise results will be over reported). The following guide is intended to give users a general protocol for titrating their beads. Cell samples should be titrated separate from the beads, and may require additional considerations not covered herein (for more on cell vs bead titration, see M. Roederer, Purdue Cyto List, 4/10/16).

PROCEDURE

A general titration involves the use of a dilution series to determine the optimal antibody concentration required for staining. Typically, the manufacturer's recommended concentration is used as a starting point, and a range of dilutions higher/lower are chosen. If desired, the highest ABC bead population may be titrated, and the determined optimal concentration used to stain all subsequent bead populations (excluding the blank).

This is a general protocol and optimization may be required. Stained beads should be analyzed on the flow cytometer immediately and at relevant instrument settings.

Materials Required

- QSC (catalog codes 815 - 817) or SC beads (catalog codes 810 - 813) specific to the antibody being used. For example, if you are using a mouse antibody, you would choose the anti-mouse kit (815 or 810).
- Fluorochrome-conjugated monoclonal IgG antibodies, again ensure that the kit is directed to the host of the antibody
- Staining buffer (PBS generally is acceptable)
- Microcentrifuge tubes
- Flow cytometer
- Centrifuge
- Vortex mixer

General Titration

1. Leave the blank be, it should not be stained with the other populations.
2. Set up 8 microcentrifuge tubes of appropriate size, with tube 1 having 100 µL of staining buffer (e.g. PBS), and 2-8 having 50 µL (figure 1).
3. Prepare an initial working dilution from the antibody stock solution in tube 1 of 4X the recommended concentration (will be further diluted to 2X after addition of 50 µl of bead solution). This should typically require a few µL of stock solution at most (no need to account for the additional volume as it will have a negligible impact)
4. Transfer 50 µL of tube 1 to tube 2, gently mixing after addition.
5. Pipet up 50 µL of solution from tube 2 and add to tube 3, again mixing after addition
6. Continue the pattern of addition/mixing for the remaining tubes (figure 1). Upon completion, remove 50 µL from tube 8 and discard the amount (all tubes should now have 50 µL total)
7. Gently shake the bottle to resuspend spheres. If needed a general microscope or flow cytometer can be used to determine monodispersity (aim for 95% or greater singlets).
8. Add 50 µL of beads into each tube, bringing the total volume of all tubes to 100 µL (this is one additional halving dilution).
9. Incubate in the dark for 30 minutes at room temperature or in a refrigerator.
10. Wash all tubes 2X (centrifuge at 2500G for 5 minutes, decant supernatant, resuspend in 500 µL of same buffer used for analysis of cell samples)
11. Conduct standard flow analysis to determine each dilution's mean fluorescence intensity (Geo Mean or Median channel value). Analyze each dilution separately, do not combine samples. If needed, refer to QSC PDS 814 & 818 for more details regarding MFI determination.

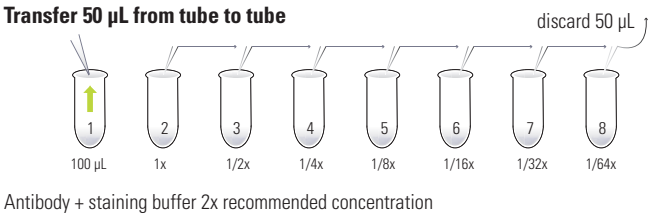

Figure 1: *Sample setup scheme for the creation of a range of dilutions to be used in an antibody titration*

Analysis of Results

Once all of the data have been compiled, a likely optimal concentration should be fairly apparent. Results should indicate a substantial increase in median channel values as the concentration is increased, up until a point, after which increased concentrations have a notably reduced impact (further rises in the median channel value can typically be attributed to non-specific binding). For instance, users may find that concentrations at 0.015x through 0.25x of recommended levels increase median channel values on average of 20% or more, but further increases to 0.50x through 2x only yield modest increases, forming a plateau.

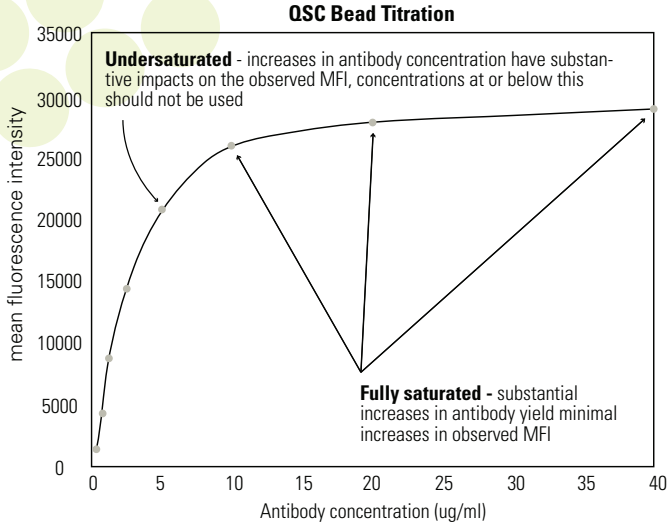

Figure 2: *Scatter plot of antibody concentration vs MFI. Appropriate saturation appears to be reached at 0.50x the recommended usage concentration*

TROUBLESHOOTING

One potential issue encountered when titrating is non-specific binding (NSB), which can cause curves to continue upward without leveling off. Users may also notice that with increasing antibody concentration that their peak histograms begin to "broaden out". In these instances, NSB may be artificially inflating the MFI of the samples, making determination of optimal working concentration difficult.

Reduction of NSB ultimately requires a personalized trial and error approach, although some good initial suggestions include[1]:

- Reducing incubation time
- Adding in additional washes post incubation
- Running "split" samples, with one set following a traditional staining, and the second set being subjected to longer/reduced incubation and additional washing
- Trying a different antibody to evaluate whether the NSB is antibody specific
- Adding some surfactant to the buffers to reduce non-specific hydrophobic interactions (use only if necessary as surfactant may also reduce specific binding)

Alternatively, poor antibody binding can be due to a variety of causes, such as sterics (large fluorochrome/antibody conjugate), antibody specificity (is the antibody appropriate for the bead anti-species IgG?), or minute differences in a recombinant antibody's Fc region (as the beads are Fc specific). Indirect staining may introduce additional considerations, such as the rate of secondary antibody binding per primary antibody, and users should note that this secondary antibody binding may differ between beads and samples due to primary antibody orientation (again due to Fc vs F(ab) oriented binding). Furthermore, labeling schemes that employ fragments of antibodies are not compatible with our QSC kit or SC microspheres, as they often lack the Fc region required for capture by the bead's anti Fc antibody. In this event, our MESF kit may be a more suitable option.

RELATED TECHNICAL LITERATURE

PDS 814 – Quantum Simply Cellular
PDS 818 – Quantum Simply Cellular & Quantum MESF Tips and Techniques

REFERENCES

1. **E Harlow and D Lane.** 1988. *Using Antibodies a laboratory manual.* pp 726. Cold Spring Harbor Laboratory. ISBN 0-87969-314-2
2. **Givan, A.L.** (2000). In Living Color: *Protocols in Flow Cytometry and Cell Sorting* (R. Diamond and S. DeMaggio, eds). Springer, Berlin, pp 142-164.
3. **Hulspas R.** 2010. *Titration of fluorochrome-conjugated antibodies for labeling cell surface markers on live cells.* Curr Protoc Cytom. Chapter 6: Unit 6.29.
4. **H. Shapiro.** 2003 .*Practical Flow Cytometry*, 4th Ed Wiley-Liss.
5. **Collino CJ, Jaldin-Fincati JR, Chiabrando GA**. 2007. *Statistical criteria to establish optimal antibody dilution in flow cytometry analysis.* Cytometry B Clin Cytom, 72:223–226.