A High Throughput Screening Homogeneous Antibody Binding Assay using the Acumen ᵉX3

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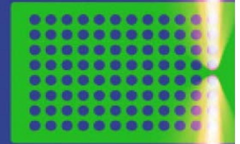


Abstract

Monoclonal antibodies are produced by hybrid myeloma or hybridoma cell lines secreting specific antibodies into the culture media. Screening of hybridoma supernatants in an homogeneous assay format have been instrumental in the development of therapeutic antibodies directed against specific cell surface targets. Here we report on the ability of the Acumen® ᵉX3 to run homogeneous assays using both beads and cells to rapidly quantify cell surface bound antibody for discovery and primary screening applications.

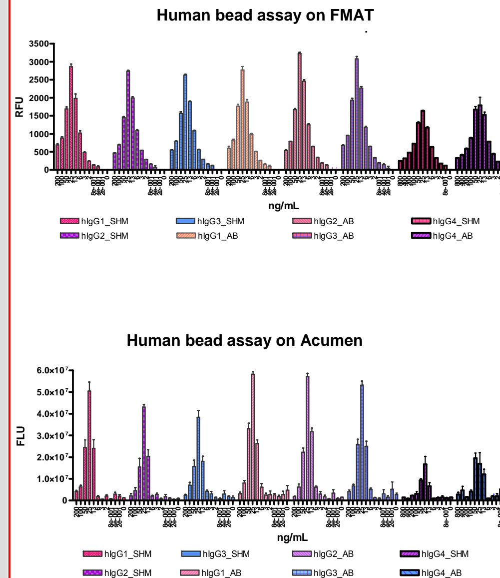
This study compares the performance of the FMAT 8100 HTS instrument with an Acumen ᵉX3 laser scanning cytometer for detecting antibody binding to cell surface markers in both bead- and cell-based binding assays.

The Acumen ᵉX3 used in this study uses triple laser (405nm, 488nm, 633nm) excitation to enable multiplexing of fluorophores. With scan times of less than ten minutes per plate, the Acumen ᵉX3 is significantly faster than multi-channel scanning using camera-based automated imaging platforms.

Conclusions

- **The trends for the quantification of cell- and bead-bound antibody are comparable between the Acumen ᵉX3 and the FMAT 8100 HTS**
- **Acumen ᵉX3 can run FMAT-type assays using the 488 nm and/or 633 nm laser line**
- **The Acumen platform offers a simple workflow, which enables fast, automated robust and reproducible homogenous antibody binding assays**

1 Comparison of Binding of Human Antibodies to Beads



Procedure:

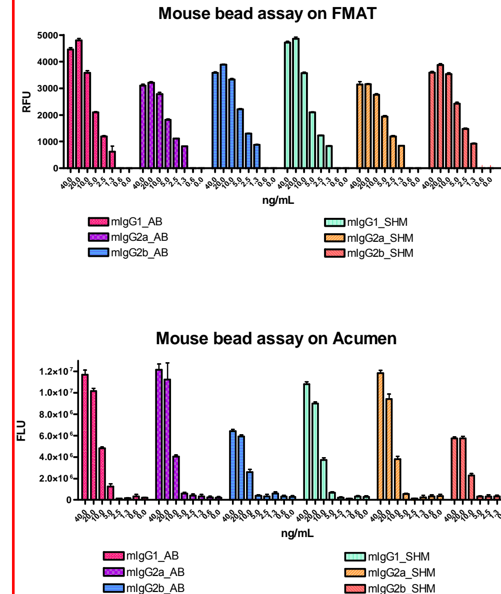
1). Human isotype controls (hIgG1−3) were prepared in assay buffer at 600 ng/mL (3X concentration). Human isotype control hIgG4 was prepared in assay buffer at 2400 ng/mL (3X concentration). The antibodies were then diluted in a 2-fold dilution series.

2). Goat anti-human Alexa Fluor® 633 conjugated secondary antibody was prepared at 600ng/mL (3X concentration).

3). Goat anti-human polystyrene beads at a concentration of 50 µg/mL were prepared in assay buffer (AB) or spent hybridoma medium (SHM).

4). Equal volumes of all three preparations described in steps 1−3 were mixed together and 50 µL was added per well into a 384 well plate. Samples were then incubated overnight prior to analysis.

2 Comparison of Binding of Mouse Antibodies to Beads



Procedure:

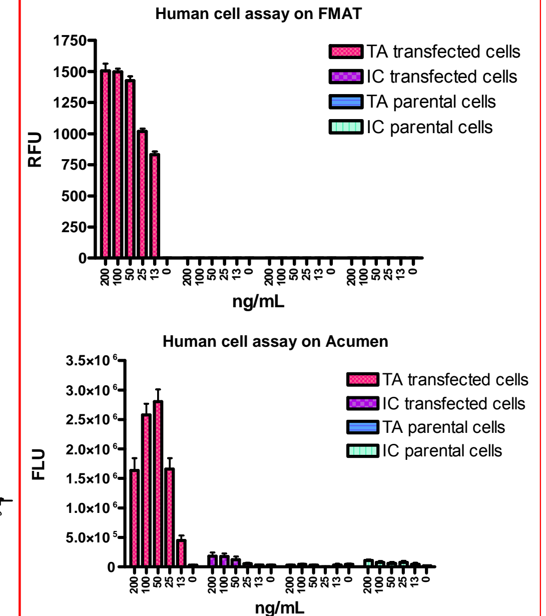
1). Mouse isotype controls were prepared in assay buffer at 120 ng/mL (3X concentration). The antibodies were then diluted in a 2-fold dilution series.

2). Secondary antibodies of goat anti-mouse FMAT blue (FMAT) or Goat anti-mouse Alexa Fluor® 488 (Acumen ᵉX3) were prepared at 600ng/mL (3X concentration).

3). Goat anti-mouse polystyrene beads at a concentration of 50 µg/mL were prepared in AB or SHM.

4). Equal volumes of all three preparations described in steps 1−3 were mixed together and 50 µL was added per well into a 384 well plate. Samples were then incubated for 1−2 hours prior to analysis.

3 Comparison of a Cell Surface Marker Detection Assay



TA = Antibody against Target Antigen, IC = Isotype Control

Procedure:

1). Goat Anti-Mouse FMAT blue at 1250 ng/mL (3X concentration) and Goat Anti-Mouse Alexa Fluor® 488 (Acumen ᵉX3) at 625 ng/mL (3X concentration) were prepared in assay buffer.

2). Transfected and untransfected cells were prepared at $2x10^5$ cells/mL (3X concentration)

3). Target antibody against target antigen and isotype control at 600 ng/mL was prepared at 3X concentration.

4). Equal volumes of all three preparations described in steps 1-3 were mixed together and 30 µL was added per well into a 384 well plate. Samples were then incubated for 1 hour at room temperature prior to analysis.