Development of a High Throughput Screening Assay that Simultaneously Quantifies Antibody Levels, Cell Number and Cell Viability.

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ABSTRACT

Targeted biologics, including monoclonal antibodies, are among the most medically important and fastest growing therapeutic modalities in the biopharmaceutical industry. Antibody development is a costly and resource intensive endeavor, and technologies that streamline the discovery process and increase productivity are greatly needed. A key process in the discovery and development phases of new antibody therapeutics lies in quantitation of secreted proteins on a per cell basis. This is typically performed using single readout assays such as ELISA or surface interference technology protein level determination, followed by separate assays that quantitate cell number. We report here a multiplexed antibody secretion screening assay that simultaneously determines antibody concentration and assesses cell count and viability in each well of a microtiter plate. To perform the assay, antibody producing cells were cultured in 96 well plates, and then a sample from each well, including cells and supernatant, was transferred to an assay plate. To determine antibody present in the samples, latex microparticles coated with an IgG capture reagent were added to the assay plate and IgG bound to the beads was detected with fluorescently tagged anti-IgG. Antibody concentration in each sample was quantified from standard curves using control reagents. In the same assay, cell count and percent of viable cells in each well were determined using a fluorescent amine-reactive dye that preferentially stains cells with compromised cell membranes. The assay plates were processed and analyzed using the HTFC Screening System (IntelliCyt). Combined with the speed of the platform (96 well plates in as little as 3 minutes, 384 well plates in 15 minutes or less), this multiplex screening assay can significantly improve productivity of the antibody discovery and development process.

INTRODUCTION

The ability to achieve high levels of antibody secretion from a clonal cells line enables sufficient quantities to be produced for research, clinical trials and drug manufacturing. Identifying cell lines that produce and secrete high levels of IgG significantly decreases capital costs associated with the production of research and therapeutic antibodies. Creation of stable recombinant cell lines is required for industrial scale production of antibodies and other engineered proteins. Clonal cell lines are generated by transfection of a population of cells with DNA encoding the IgG genes of interest. This procedure results in a population of transfected cells with the recombinant DNA inserted into many different chromosomal sites in different cells within the population. Some cells within this mixed population produce and secrete IgG molecules at low levels, some at moderate levels and a few at very high levels. Determining which clones will yield the highest levels of IgG is of critical importance to the success of clinical and commercial applications. In general, there is a correlation between clones that secrete the highest levels of IgG and clones that secrete IgG at the fastest rate. Screening methods that examine either of these parameters can thus be used to identify the most productive clones for downstream processing.

ELISA assays are generally used to screen transfected cell clones by determining the amount of IgG present in the supernatants of individual wells in microtiter plates in which the cells have been cultured. Once supernatants that contain high levels of IgG have been identified, a second process is initiated in which the cells from which those supernatants were generated are cultured to determine their rate of growth. This is an expensive and time consuming process. Described in this work is a screening method which combines a bead based ELISA assay with a cell based assay to simultaneously measure the amount of IgG present and the number of cells in each well of a microtiter plate. In addition the assay can be adapted to included viability information for each cell in the population

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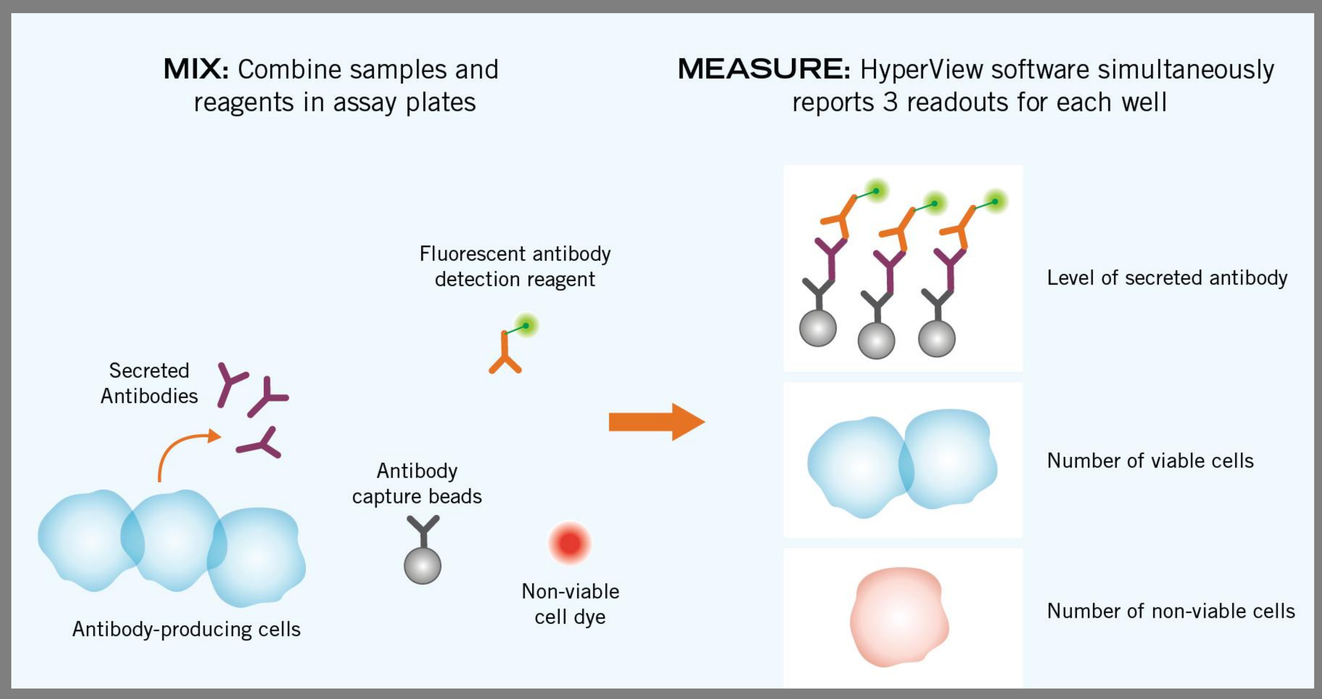
ASSAY CONCEPT



Components and workflow of the IntelliCyt screening assay for simultaneously detecting antibody secretion, cell number and cell viability. Antibody secreting cells are mixed with assay reagents, including capture beads, a detection reagent and a cell viability dye. Using the HTFC Screening System, the level of antibody present in the supernatants, the number of cells and the number of non viable cells is reported.

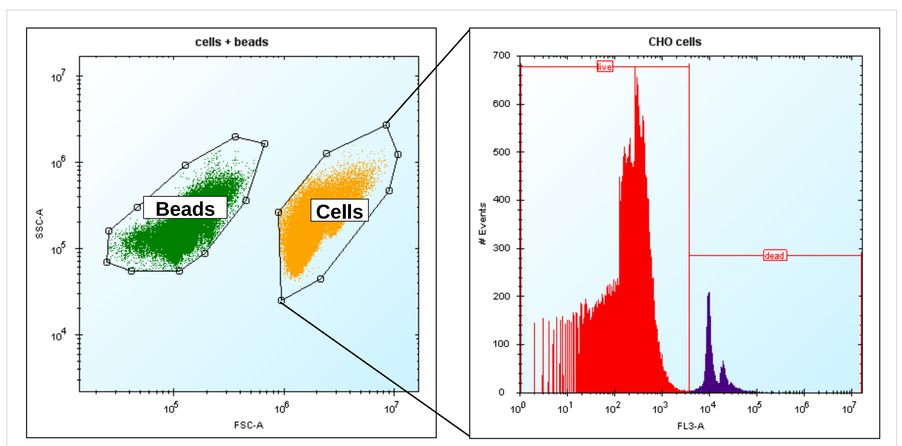
HTFC® SCREENING SYSTEM

CONCLUSIONS

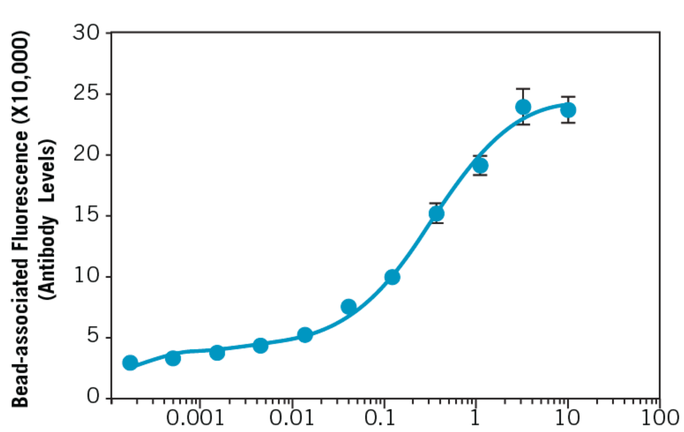
A multiplex screening assay is presented that simultaneously measures IgG levels, cell number and cell viability in each well of microtiter plates.

- The assay utilizes the HTFC Screening System, and combines two assays in each well:
  - A bead based ELISA assay in which IgG molecules are captured and detected on microparticles.
  - A cell based assay in which the number of cells is counted and the viability of each cell is determined.
- The ratio of IgG signal to cell number provides a meaningful description of clones that are capable of secreting high levels of IgG.
- It is possible to perform this screen in a kinetic fashion, which would enable the determination of specific productivity over time.

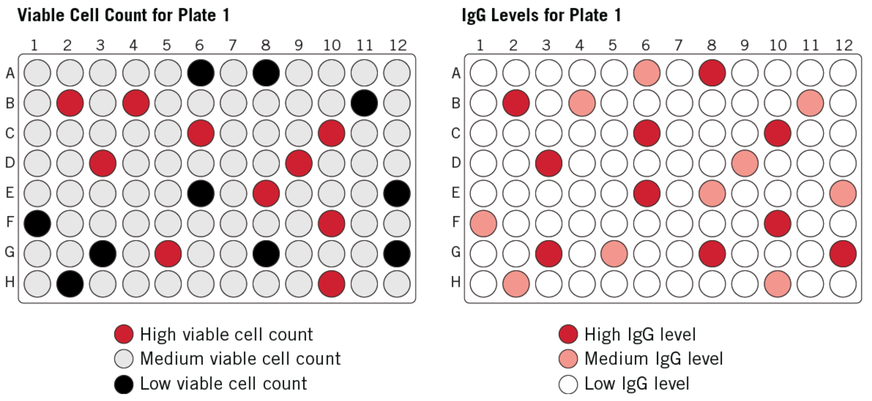
SCREENING ASSAY DATA



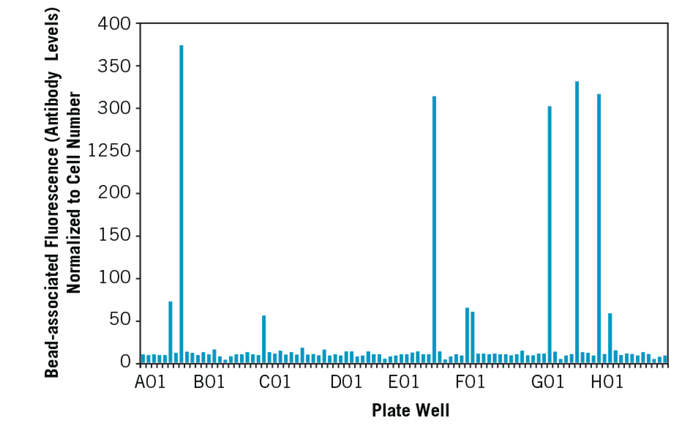
Simultaneous detection of IgG capture beads and IgG producing cells. All beads and cells from the entire plate are depicted in the graphs below. The beads and cells are easily distinguished based on forward scatter properties (left panel). Live and dead cells are distinguished in the FL-3 channel (right panel). Fluorescent antibody bound to the beads is detected in the FL2 channel (not shown).



Dynamic range of IgG detection. Serial dilutions of IgG were tested using the assay protocol. Results show that the assay can accurately detect IgG input concentrations in the nanogram to microgram range.



Identification of hit scenarios from screening plates. Heat maps from a single assay plate run on the HTFC Screening System showing two different readouts for each well of the assay.



Graph showing IgG signal normalized to cell number for each well of the plate. Using this approach, clones with high IgG production on a per cell basis can be easily identified.

REFERENCES

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