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Multiplexed Screening for Antibody Production

A Novel Multiplexed Screening Assay for Assessing Productivity of Antibody-Secreting Cell Lines: Simultaneous Measurement of Secreted Protein and Viable Cell Count

Key Features & Benefits

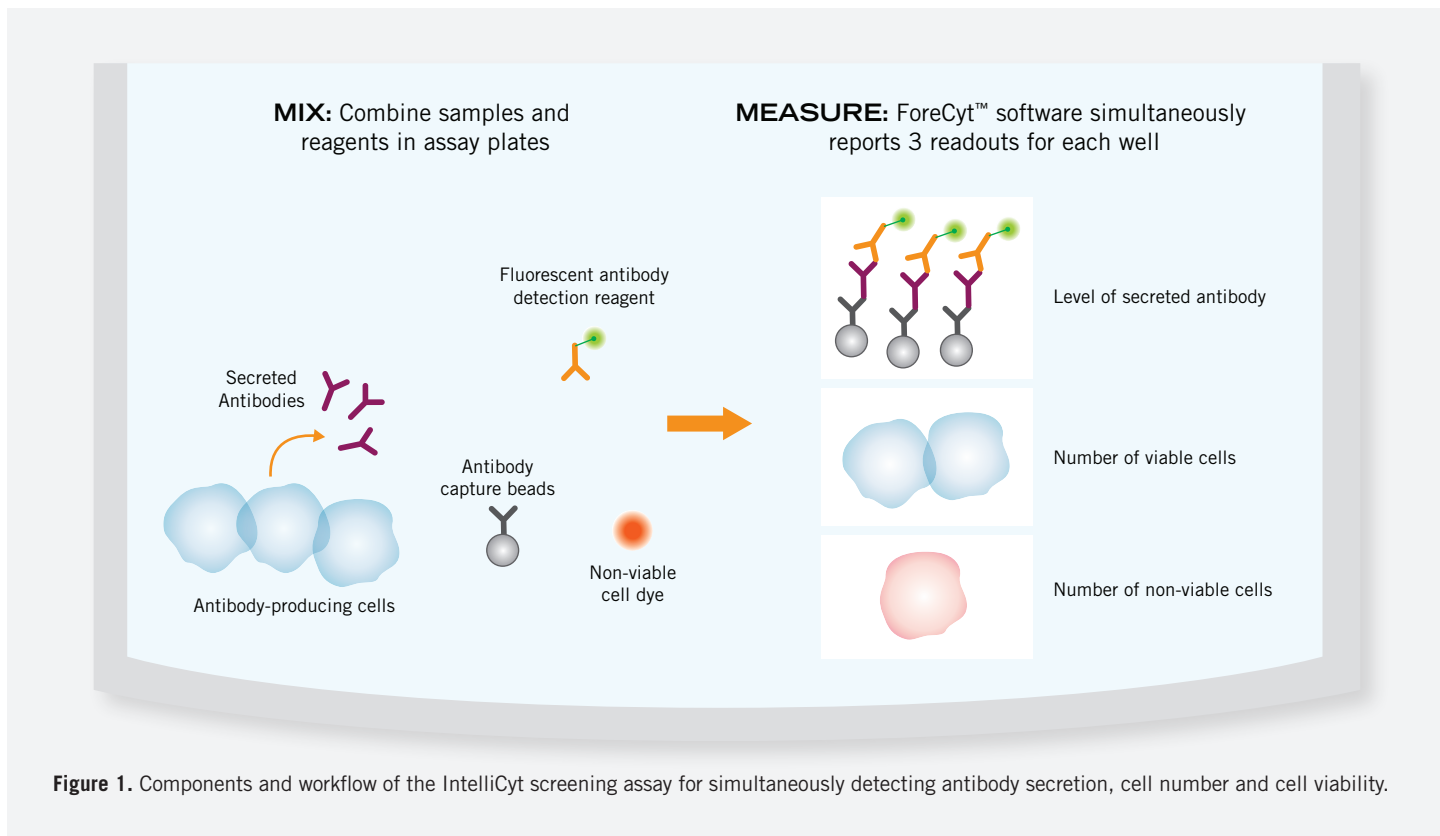| FEATURES	BENEFITS
• Multiplexed bead and cell format	• Simultaneous quantification of secreted protein and viable cell count
• Large dynamic range (ng to mg per mL)	• Reduced dilution and processing steps
• Single-wash assay	• Minimized assay time, cost, variability

Introduction

Monoclonal antibodies (MAbs) and related products are a dominant component of the biopharmaceutical market and are being used to treat diseases such as cancer and inflammation, and generating revenues of several billion dollars each year (1). A key component of the development of a therapeutic monoclonal antibody is the identification and selection of a manufacturing cell line that secretes high levels of the antibody into the culture supernatant. Finding a highly productive cell line is critical to the success of a potential antibody based therapeutic, because the downstream optimization and scale-up processes can be exceedingly time intensive and costly. Early identification of high producing cell lines can significantly increase the probability of success in downstream scale up activities (2, 3). The most commonly used methods for assessing productivity involves the generation and growth of a clonal library of production cell lines followed by a high throughput assay that measures the amount of secreted protein in the supernatants from each clone. These assays can be based on ELISA or surface interference technologies, which, while robust and quantitative, are single readout assays (3). and cannot distinguish between highly productive cell lines, or the result of a fast growing, low producing cell line. Thus hits from a screen using these single readout assays must be followed by secondary assays aimed at assessing the amount of antibody secreted on a per cell basis. We present here a novel multiplex assay for IntelliCyt platforms that simultaneously reports antibody levels; cell number and cell viability in each well for cell line production screening plates (see **Figure 1**).

Assay Principle

Samples containing engineered cells secreting antibodies were mixed with anti-human IgG capture beads and incubated with shaking for 30 minutes at room temperature. Plates were centrifuged, washed once with phosphate buffered saline (PBS) and samples resuspended in PBS containing the fluorescently labeled detection reagent and incubated for 30 minutes at room temperature. Finally, the nonviable cell dye was added to the wells and the plates were incubated for 15 minutes. Plates were directly

MIX: Combine samples and reagents in assay plates

MEASURE: ForeCyt™ software simultaneously reports 3 readouts for each well

Fluorescent antibody detection reagent

Secreted Antibodies

Antibody capture beads

Non-viable cell dye

Antibody-producing cells

Level of secreted antibody

Number of viable cells

Number of non-viable cells

Figure 1. Components and workflow of the IntelliCyt screening assay for simultaneously detecting antibody secretion, cell number and cell viability.

sampled and the data generated was analyzed with the HTFC® Screening System.

Secreted antibodies in the samples bind to the capture beads, and are detected by the fluorescently labeled detection reagent. A dye that specifically labels non viable cells is included to determine the ratio of viable to nonviable cells. When plates are sampled on the HTFC Screening System, three readouts are simultaneously reported: 1) Antibody levels in the well, 2) Total cell number and 3) Percent of nonviable cells in each sample. A ratio of antibody levels to cell number is easily calculated.

Results & Conclusions

To simulate an antibody producing clone selection environment an experiment was set up in which CHO suspension (CHO-S) cells plus known concentrations of human IgG were added to the wells of assay plates and processed according to the protocol above.

The antibody detection range for the assay was determined by preparing plates with serial dilutions of human IgG. Input concentrations starting at 10 mg/mL were serially diluted 1:2 and a constant number of CHO-S cells were added to each well. At the start of the assay, each well contained 20 µL IgG and 20 µL cells plus 10 µL capture beads. The assays were performed in quadruplicate. **Figure 2** shows the IgG levels present in the samples, as represented by the bead associated fluorescence. The data demonstrated that the assay can easily detect and differentiate 10 mg/mL to 10 µg/mL input IgG levels with minimal dilution of the starting sample.

To demonstrate the utility of the assay and the HTFC Screening System software in a screening environment, certain wells were set up in plates to mimic different scenarios that might be observed in a screening campaign:

1. High IgG level / High cell number

2. High IgG level / Low cell number

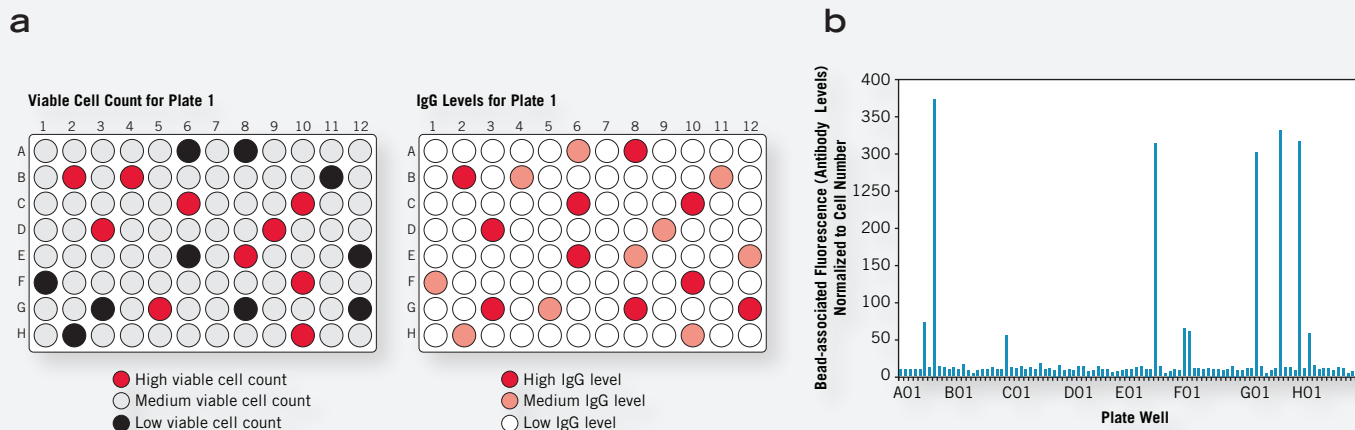3. Low IgG level / High cell number

4. Low IgG level / Low cell number.

Figure 2. Range of IgG detection.
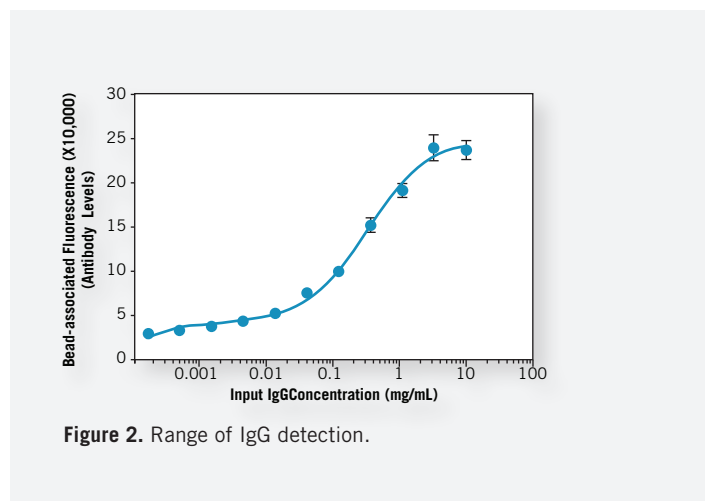
Figure 3. Identification of hit scenarios from screening plates. **A.** Heat maps from a single assay plate run on the HTFC Screening System showing two different readouts for each well of the assay. **B.** Graph showing IgG signal normalized to cell number for each well of the plate.

Figure 3A shows that using the HTFC ForeCyt software, heat maps can be generated to easily differentiate between the scenarios. By customizing the color coding of the wells of the heat maps, picking out the optimal scenario, where the IgG level is high and the cell numbers are low is simplified. For example, wells B2 and A8 both have high IgG levels (red), but B2 has a high number of cells, and A8 has a low number of cells. This would indicate that well A8 has cells with higher productivity.

To further quantify the relative productivity of the cells in each well, the IgG levels can be normalized to the cell number in that well. **Figure 3B** shows a plot of each well of a 96-well plate in which the fluorescence level that was associated with the capture beads in each well was divided by the number of cells in each well. Using this approach, one can easily set criteria to identify wells containing clones that produce high levels of antibody on a per cell basis.

Summary

In this report we demonstrate a novel multiplex assay for the HTFC Screening System that provides antibody levels on a per cell basis by combining the ability to measure secreted antibody levels, cell count and cell viability into one assay. In a cell line generation setting where libraries of single cell antibody secreting clones are screened and assessed for productivity, this screening assay could significantly improve the chance of success of identifying high producers for further downstream analysis.

References

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2. Farid S. Process Economics of Industrial Monoclonal Antibody Manufacture. *J. Chromatogr.* B 848, 2007: 8–18.

3. Birch JR, Racher AJ. Antibody Production. *Adv. Drug Del. Rev.* 58, 2006: 671–685.