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

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 highly productive 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 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 highly productive 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 selection of a manufacturing cell line that secretes high levels of the antibody into the culture supernatant. 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

Key Features & Benefits

**FEATURES**
- Multiplexed bead and cell format
- Large dynamic range (ng to mg per mL)
- Single-wash assay

**BENEFITS**
- Simultaneous quantification of secreted protein and viable cell count
- Reduced dilution and processing steps
- Minimized assay time, cost, variability
**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.
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 identifying high producers for further downstream analysis.

References
