A Novel Clone Ranking and Selection Assay that Combines all the Relevant Insights Needed to Choose the Most Optimal Clones in a Single Experiment

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Abstract

When developing cell lines for the production of antibodies, it is essential to choose healthy clones with high levels of IgG per cell. This process typically requires several assays on multiple platforms to get enough information to select optimal clones. Due to the expense and effort involved, this limits the number of clones that can be evaluated. We have developed the Cy-Clone™ PLUS Kit, a rapid, no-wash screening assay that simultaneously provides IgG concentration, IgG concentration per cell and per viable cell, cell viability, and cell density in every well of a 384-well plate.

CHO cells transfected with human IgG genes were inoculated, plated by limiting dilution, and cultured for 20 days. Cells were mixed with the reagents from the Cy-Clone PLUS Kit and then read on the iQue® Screener PLUS platform in ~22 minutes. Using ForeCyt® Software, readouts for each well were visualized using heat maps and Profile Map to identify wells meeting multiple IgG production criteria.

The Cy-Clone PLUS kit with the iQue Screener platform provides all the relevant data needed to select and rank the best clones in a single well without screen-size limitations. The Cy-Clone PLUS kit enables you to make more informed decisions earlier, shorten development time, and decrease costs.

Background

Finding a highly productive cell line early in monoclonal-based drug development 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. The most commonly used methods for assessing productivity involve 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 and cannot distinguish between highly productive cell lines or the result of a fast growing, low producing cell line. Consequently, 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. Presented here is a high throughput cell-based assay that simultaneously evaluates the amount of secreted antibody and the number and health of each cell secreting the antibody.

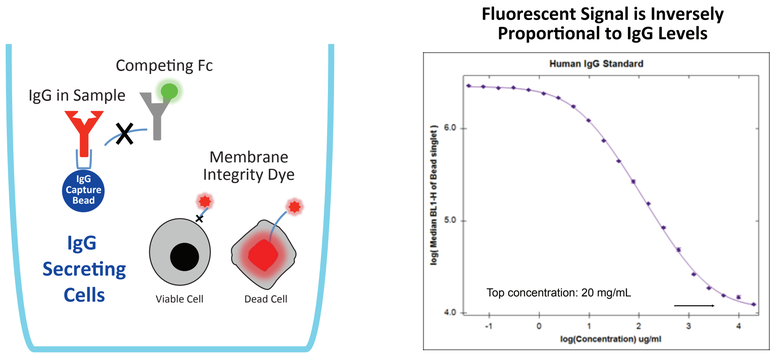

Figure 1: Principle of the Cy-Clone Kit. The Cy-Clone PLUS Kit is a no-wash assay that enables the simultaneous quantification of secreted IgG per cell and per viable cell from each well of a screening plate. Fluorescently-labeled IgG (FL-IgG) is added to samples containing secreted IgG and CHO production cells. The FL-IgG and unlabeled sample IgG compete for binding to IgG capture beads. The amount of IgG present in the sample is inversely proportional to the bead-associated fluorescence. Cell viability is simultaneously measured in each well using cell membrane integrity dyes, fluorescent molecules that are cell impermeant. Healthy cells with intact cell membranes exclude the dye and are not fluorescent. Unhealthy cells with compromised membranes will allow entry of the dye into the intracellular space, where it then localizes to the nucleus and binds to DNA by intercalation.

A standard curve from the assay shows a wide dynamic range (right panel). Twofold dilutions of an IgG control standard were tested and demonstrated a dynamic range of 0.6 µg/mL to 20 mg/mL.

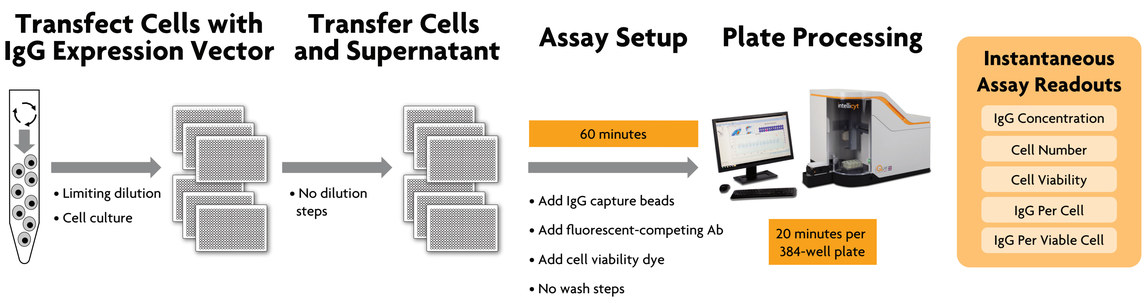

Figure 2: Screening Workflow. IgG secreting cells were distributed into culture plates by limiting dilution and cultured for 20 days. After growth, 20 µL samples from each well (including both cells and supernatants) were transferred to assay plates and mixed with kit reagents. After incubation at room temperature for 60 minutes, plates were read on the iQue Screener PLUS, with instantaneous readouts by ForeCyt Software, up to 76% faster than with legacy methods.

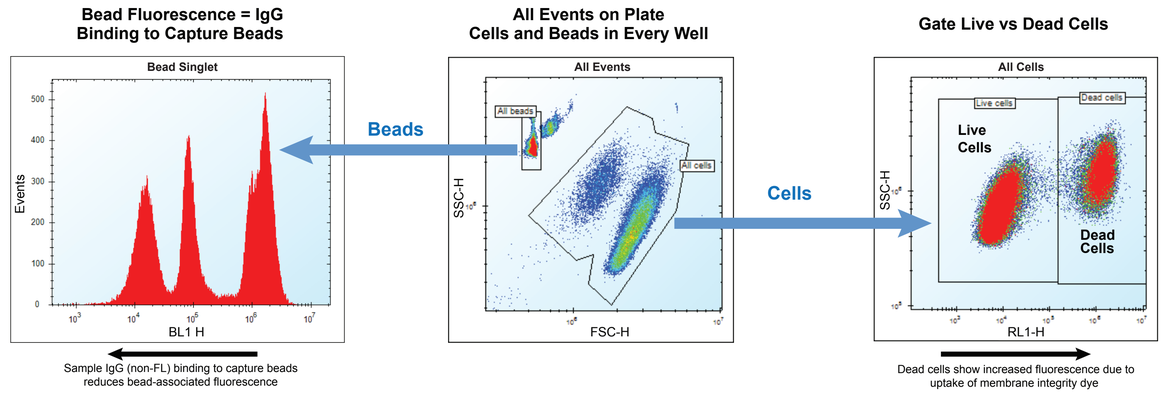

Figure 3: ForeCyt Software automates the data acquisition and analysis of the cell- and bead-based assay. Cells and beads were first identified and distinguished based on size (center panel). Fluorescence intensity of the capture beads was determined, and was inversely proportional to the quantity of captured IgG (left panel). Dead cells were detected by increased fluorescence associated with uptake of the cell membrane integrity dye (right panel).

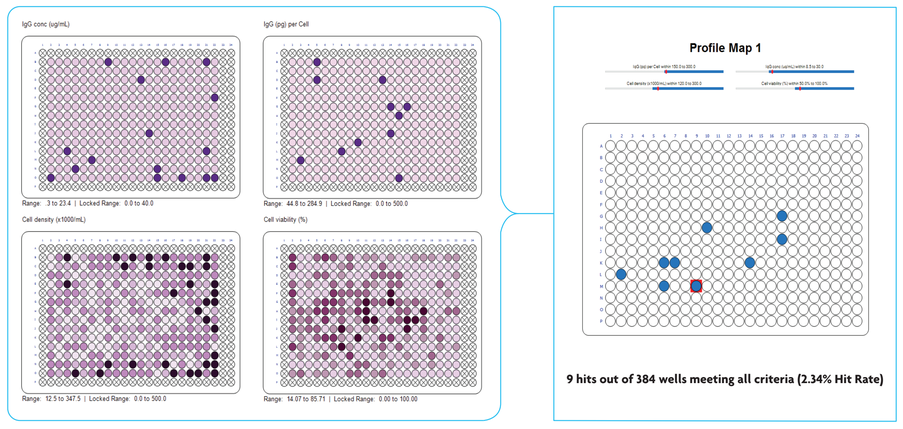

Figure 4: ForeCyt Software combines multiple parameters to rapidly identify wells containing highly productive IgG secreting cells. Data from Plate 3 of the screen are shown here. Four heat maps from the plate provided a visual representation of: i) IgG concentration; ii) amount of IgG per cell; iii) number of cells; and iv) cell viability. The profile map feature uses Boolean logic to combine multiple parameters into a single readout for each well. Hits were selected based on meeting user-defined criteria for IgG concentration, amount of IgG per cell, number of cells, and cell viability simultaneously.

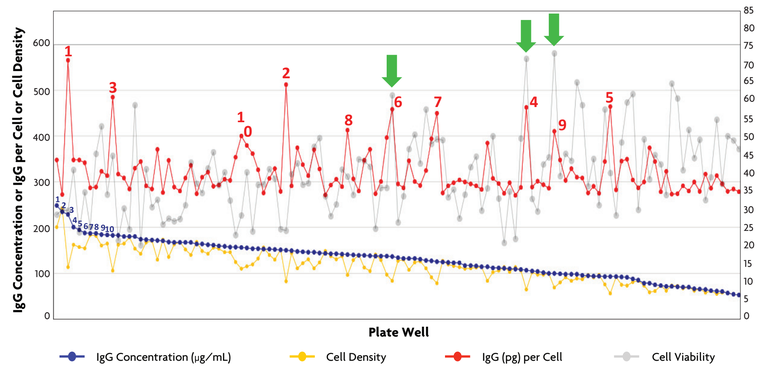

Figure 6: IgG production on a per-cell basis identifies better hits. The top 124 hits from the screen, based solely on IgG concentration, were plotted in decreasing order (blue line). The top 10 ranked hits based on this criterion are shown. The cell number in each of the hits is shown with the yellow line. Combining IgG concentration and cell number to calculate IgG per cell (red line) identified a very different ranking of the top ten hits, and provided a more robust indication of productive cells. Overlaying cell viability data enabled the identification of hits which exhibit both high levels of IgG productivity per cell and cell health.

Summary and Conclusions

Demonstrated here is a novel, robust screening assay for the determination of cell line productivity utilizing the iQue Screener PLUS platform and the Cy-Clone PLUS Kit. In each well of screening plates, the amount of secreted IgG per cell was determined and compared to the number and viability of cells in each sample, providing rich information on cell health and cell growth during the culture period. In a cell line generation setting in which libraries of single-cell, antibody-secreting clones are screened and assessed for productivity, this assay significantly improved the chance of success of identifying high producers and minimized the need for a second cell-based assay, reducing the time and cost required to conduct the screen.

Advantages of the Cy-Clone PLUS Kit and iQue Screener PLUS Platform for Cell Line Generation Screening

- Cy-Clone PLUS kit provides IgG concentration, cell density, IgG per cell, and cell viability in a single assay on a single platform versus correlation of data from different assays.
- Cell ranking and selection balances these direct readouts to easily and quickly identify optimal clones.
- Cy-Clone PLUS is a single, cost-effective assay that can migrate through the cell line development workflow from screening to scale up.
- Cy-Clone PLUS sample-to-answer time is up to 76% faster than with legacy methods, providing answers in 80 minutes—60 minutes of a no-wash assay setup and 20 minutes for acquisition of a 384-well plate and instantaneous readouts.
- Cy-Clone PLUS has a large dynamic range (ng to mg per mL), enabling the transfer of cell culture samples directly into assay plates without the need for dilution steps.

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