

Measurement of antibody secretion from individual cells for biomanufacturing

Introduction

Generation of stable cell lines secreting high levels of antibody or other protein is a challenging problem and represents a major bottleneck in biomanufacturing. Biomanufacturing in mammalian cell lines entails significant effort in identifying and selecting highly secreting clones, and determining optimal media and culture conditions for maintaining high secretion.

There is significant need for a sensitive assay for monitoring antibody secretion from large numbers of samples. Bulk measurements (e.g., ELISA and HPLC) suffer from key drawbacks including limited sensitivity and inability to rapidly measure large numbers of small samples. Bulk measurements also provide no insights about the dynamics and heterogeneity of the cell population. Monitoring secretion heterogeneity and changes within the cell population during the optimization processes provides better decision making criteria resulting in shortened timelines and more robust productivity.

This application note presents a novel, patented assay, based on *in situ* measurement of specific protein secretion at the individual cell level, which provides greater sensitivity than conventional assays. In addition, Cytellect's Cell Secretion Assay quantifies the amount of antibody secreted by each individual cell within a population, enabling assessment of the distribution of antibody secretion within a population of cells.

Approach and Results

The *in situ* Cell Secretion Assay employs a capture agent on the bottom of a plate specific to the secreted antibody. Upon culture of the cells on this surface, a proportion of the secreted antibody is captured on the surface near the cell. Cells are incubated for a period of time, washed, and then stained for the specific secreted antibody and with a live cell viability stain. The secreted antibody around each cell can easily be visualized and quantified (Fig. 1) (Hanania et al. 2005).

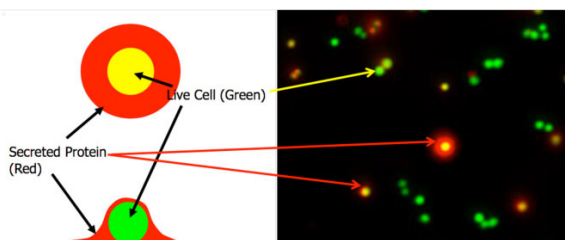


Fig. 1. *In situ* Cell Secretion Assay. All live cells (green) are stained with a viable cell dye. Secreted protein is captured local to each specific cell (red).

A range of cell types have been analyzed using the Cell Secretion Assay, including recombinant CHO, NS0, hybridoma, as well as primary B cells. Secreting cells are typically imaged after an overnight incubation, however secretion has been measured in as little as 3-4 hr for very highly secreting cells. This assay is adaptable to cells whether they are non-adherent or adherent, cell lines or primary cells.

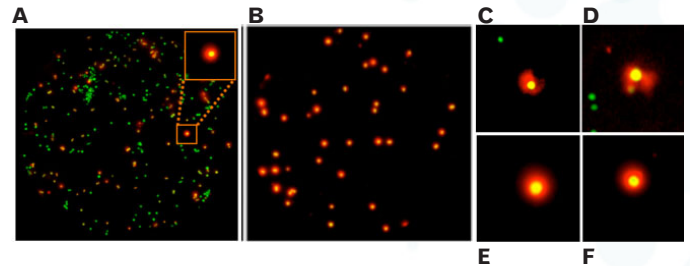


Fig. 2. Quantitative Cell Secretion Assay for characterizing biopharmaceutical cell cultures. (A) Pool of heterogeneous IgG secreting suspension-adapted CHO cells. Cells were identified by green fluorescence. Cell-associated IgG secretion was identified by red fluorescence halos around cells. (B) Homogeneous cell line population after ~50 doublings from clone isolated using CellXpress™ (Cytellect, Inc. see Application Note: CellXpress™ powered by LEAP - Accelerated Development of Highly-Secreting Cell Lines). (C-D) Primary mouse B cells secreting antibodies specific to an experimental vaccine epitope. (E-F) Mouse hybridoma.

The Cell Secretion Assay provides quantitative assessment of secretion on small cell samples (Fig. 2). Comparing standard bulk HPLC measurements with the *in situ* Cell Secretion assay demonstrates excellent correlation (Fig. 3). Importantly, sensitivity with the Cell Secretion Assay is greater, enabling quantitative measurement of secretion on clonal samples well before there are sufficient numbers of cells for bulk assays. This early assessment can be used to quickly eliminate cultures that are not going to be of value. The approach also provides a more rapid assessment of large numbers of samples, for example, during optimization protocols. Small samples of cultures can be arrayed into 384W plates and assayed in parallel. The quantification of secretion levels early in the cell line development process enables a very early ranking of samples and elimination of those that clearly fall below an acceptable threshold (Fig. 4), leading to significant cost savings in both material and personnel time.

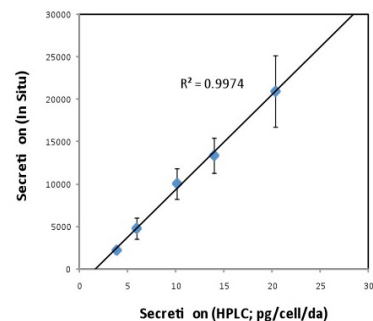


Fig. 3. Quantitative comparison of Cell Secretion measurement and HPLC. Several clonal cell lines secreting differing levels of IgG were analyzed by both Cell Secretion and conventional HPLC. *In situ* secretion measured by Cell Secretion was highly correlated with HPLC measurement from supernatant. Cell Secretion values represent the average secretion per cell in each sample.

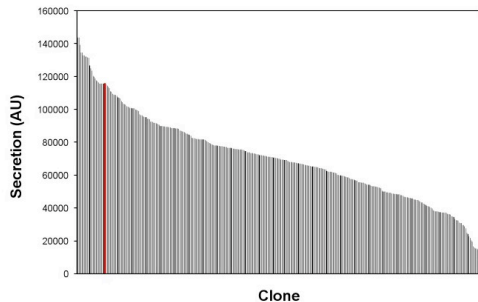


Fig. 4. Ranking of biopharmaceutical clones. Clones, isolated by CellXpress™ were ranked by assessing secretion using the Cell Secretion Assay while single clones. Red bar indicates a standard cell line producing 20 pg/cell/day of human IgG.

Visualization and quantification of secretion from individual cells facilitates exploring details about the clonal population during outgrowth (Fig. 5). Some clones exhibit the expected Gaussian-like distribution whereas others exhibit a much more skewed distribution. This insight into the population distribution may contribute to better culture optimization processes compared to relying only on assessing bulk production of protein.

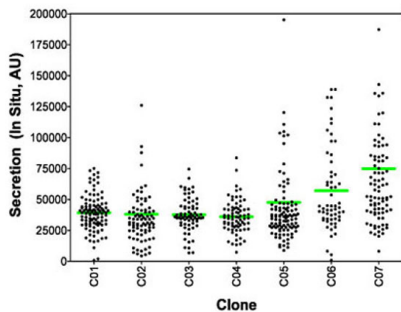


Fig. 5. Distribution of secretion within clonal populations. Clonal populations can exhibit a range of distribution shapes. Some are more normally distributed (C1-C4) where as others exhibit different degrees of skew (C5-C7). Green bar represents the average secretion value of the population. Each dot represents the secretion value from a single cell in a 384 well.

The Cell Secretion Assay provides detailed cell-level information that can be correlated with secretion. There is little correlation between secreted antibody and cell-associated antibody (Fig. 6A). In fact, the highest

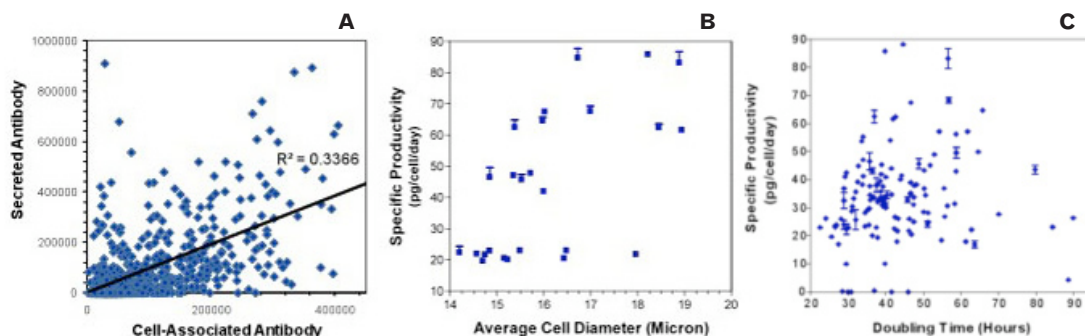


Fig. 6. Characteristics of cells vs. secretion levels. The Cell Secretion Assay has highlighted several cell-level features that may impact protein production. (A) Measurement of cell-associated antibody is insufficient to identify the highest producing cells (Hanania et al. 2005). (B) Often the highest secretors are the largest cells; however, (C) the highest secretors do not necessarily have the best growth characteristics.

secretors often have the least amount of antibody associated with the cell. Larger cells tend to be the higher secretors (Fig 6B), but also tend not to have the best growth characteristics (Fig 6C).

Conclusions

The *in situ* Cell Secretion Assay is a sensitive secretion assay as well as a powerful tool for assessing detailed characteristics of the best antibody secreting cell populations. This insight into characteristics of the secreting cell populations, at the individual cell level, opens up opportunities for selection of better clones as well as better production conditions. The application provides accurate assessment of clone secretion in the earliest stages of cell line development when only small numbers of cells are available, enabling early decisions on clone quality and eliminating effort wasted on non-productive clones. The Cell Secretion Assay on Celigo has proven to significantly reduce the time and effort in identification of cells for biopharmaceutical manufacturing.

References

- Hanania et al., Biotech. and Bioengineering, 91(7), 2005.

About the Celigo Adherent Cell Cytometer

The Celigo adherent cell cytometer enables *in situ* brightfield and fluorescence analysis of adherent cells with minimal sample manipulation. The system allows for the measurement of multiple cell-based parameters. The system enables cell biologists to:

- Analyze cell cultures *in situ*, with minimal disturbance
- Eliminate invasive enzymatic disruption during sample preparation
- Image cells in brightfield and fluorescence on the same platform
- Analyze every cell in every well with no 'edge effect'

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