Application Note 01

Rapid and efficient screening of individual IgG-secreting mammalian cells to find rare, high-producer clones

This Application Note demonstrates how Cyto-Mine® analyses large heterogeneous cell populations on a cell by cell basis using a miniaturised, homogeneous, animal origin free (AOF) IgG secretion assay to measure and identify the highest antibody-producing clones.

Introduction

The primary goal of Bioprocess Cell Line Development is to obtain high product yields from robustly-growing and well-defined clonal cell lines in timelines measured in weeks rather than months. A substantial bottleneck in the process is screening for and isolating rare cells with the required characteristics. Traditionally, this was achieved by the resource-intensive method of limiting dilution, and more recently aided by semi-automated technologies such as cell sorting, colony picking and cell-in-well imagers.

The Cyto-Mine® Single Cell Analysis and Monoclonality Assurance System overcomes the limitations of current technologies by screening hundreds of thousands of individual cells for secreted target protein, and then isolating and dispensing the highest producers with high viability to microplate wells (**Figure 1**).

The present study illustrates the process by which the Cyto-Mine® IgG secretion assay measures the production rate of hundreds of thousands of single cells encapsulated in highly consistent picoliter 'test tubes'.

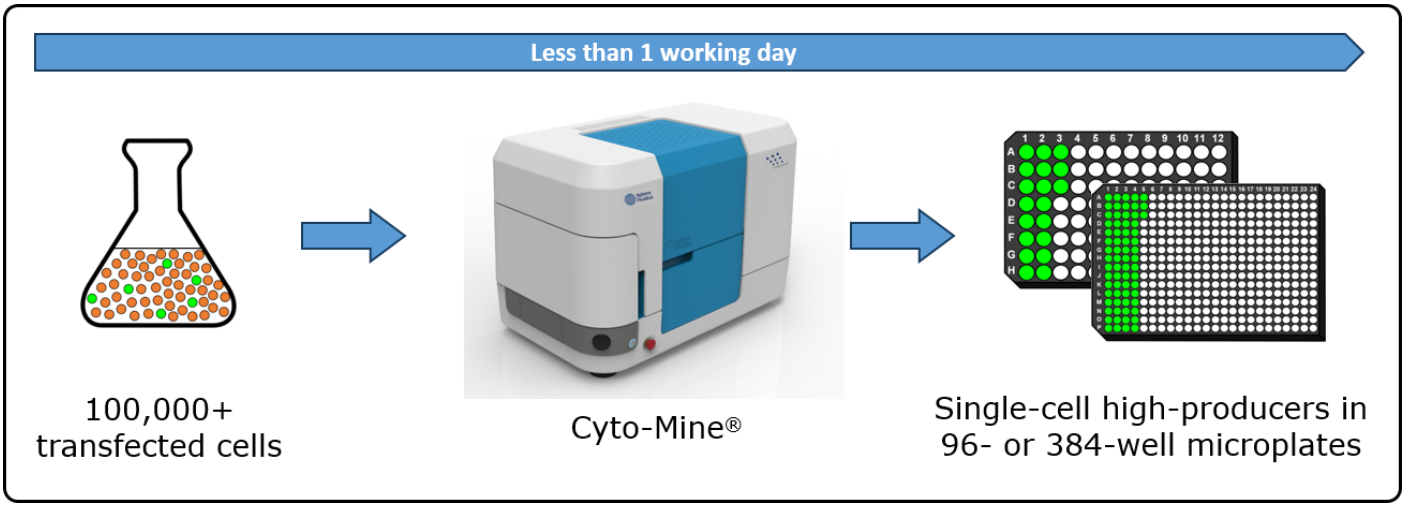

Figure 1. Cyto-Mine® proprietary technology finds and isolates high-producer clones from complex cell populations.

Methods

Cyto-Mine® emulates the limiting dilution and productivity screening process but in a much more efficient, higher throughput and fully automated manner. Using Poisson distribution statistics, cells are encapsulated into 300pL picodroplets of preferred culture medium at a dilution level that optimizes the number of picodroplets containing only a single cell. Next, the cells are incubated to enable the secreted target protein to accumulate inside the picodroplet, which is captured and detected by AOF IgG detection reagent present in the culture medium. The miniaturized scale means that secreted IgG can be quantified after just 0.5 to 2 hours. The highest-secreting single cells are then sorted for collection. The integrated stages of the Cyto-Mine® process are summarized in **Figure 2**.

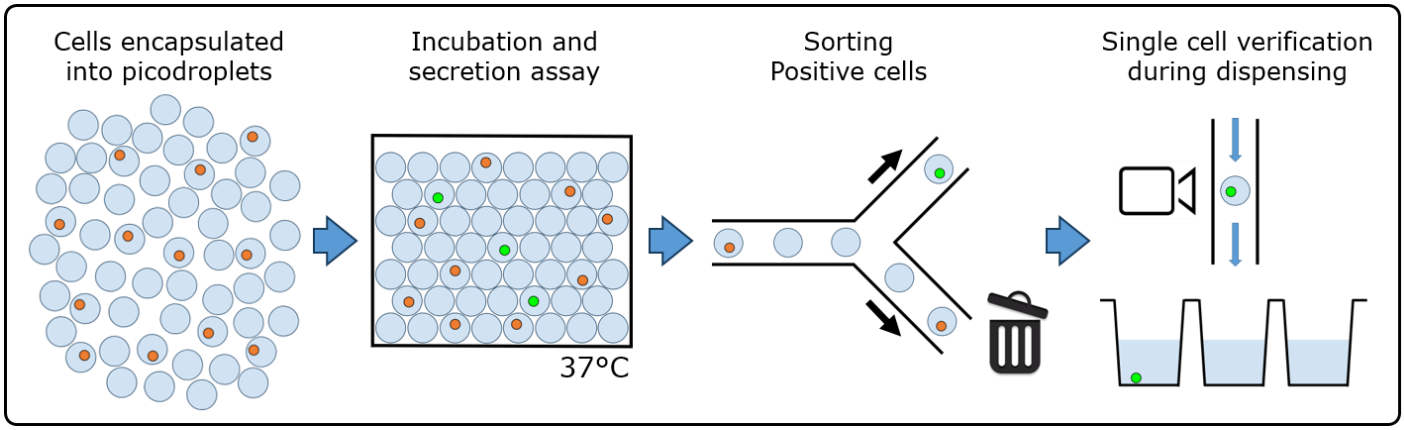

Figure 2. The Cyto-Mine® workflow integrates the screening, sorting, isolation and verification of high-secreting clones into a fully automated process.

Cyto-Mine® IgG secretion assay

A key enabling component of Cyto-Mine® is its ability to measure the specific IgG production rate of every single cell. The starting cell population requires no prior modification, and is simply mixed with the appropriate AOF detection reagent prior to loading on to Cyto-Mine®. During the *in situ* incubation step, IgG secreted by the cell accumulates within the picodroplet. The detection probes bind to the secreted IgG inducing a FRET-mediated shift in fluorescence (**Figure 3**). Cyto-Mine® then measures the fluorescent signal generated and converts it to a quantitative output.

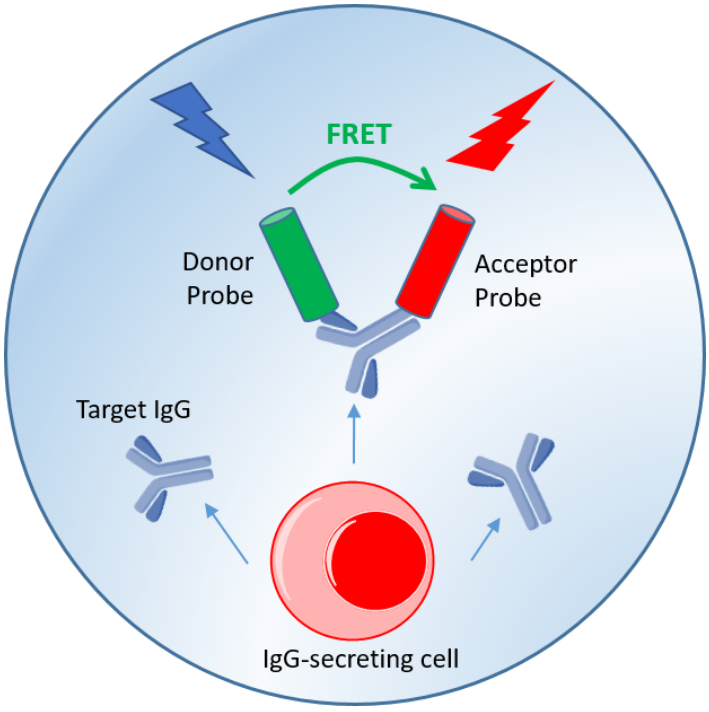

Figure 3: The Cyto-Mine® picodroplet-based IgG secretion assay. A customized pair of IgG-specific AOF fluorescent probes are trapped within each picodroplet. IgG secreted from the encapsulated cell is recognised by the detection probe pair forming a 3-body FRET complex that induces a fluorescent signal.

Results

IgG standard titration curve

To validate the Cyto-Mine® IgG secretion assay, 5 separate populations of picodroplets were generated from culture medium spiked with human IgG over a range of concentrations from 0 to 20mg/L. The 5 picodroplets populations were then pooled together and analysed using Cyto-Mine® AOF technology. **Figure 4** shows how the different titers resolved into discrete populations. IgG concentrations up to 20mg/L represent the typical Cyto-Mine® working range, equivalent to a specific productivity (Qp) of up to 144pg/cell/day assuming a 1-hour incubation. A standard titration curve generated from Cyto-Mine® Scatter Plot data is shown in **Figure 5.**

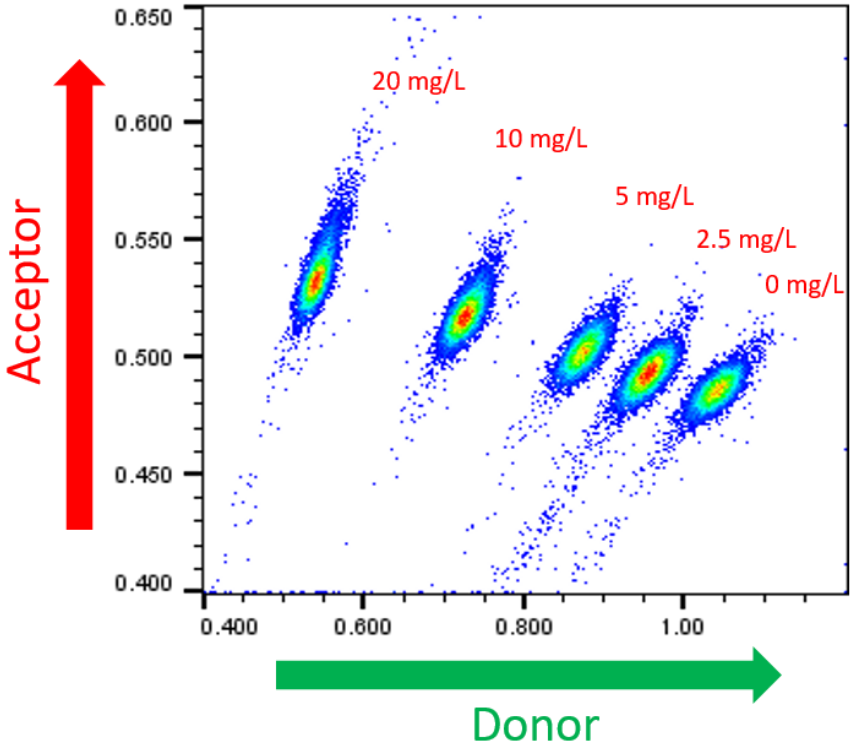

Figure 4: Cyto-Mine® Scatter Plot. Large numbers of individual picodroplets were loaded with the indicated concentrations of human IgG and then resolved using Cyto-Mine® AOF IgG secretion assay and analysis.

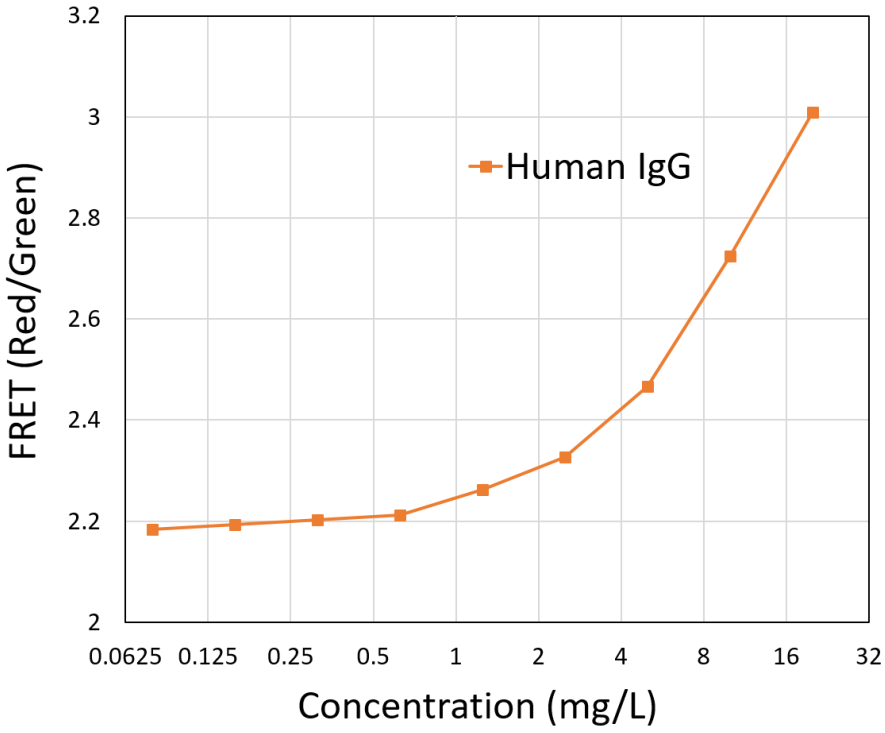

Figure 5: Standard human IgG titration curve derived from Cyto-Mine® AOF IgG secretion assay and analysis.

Screening a CHO cell population for high-producers

A heterogeneous pool of CHO cells stably transfected to express human IgG was mixed with Cyto-Mine® human IgG-specific AOF detection reagent. The cells were encapsulated into picodroplets, incubated for 2 hours, and then analysed using the Cyto-Mine® IgG secretion assay. The results, presented in **Figure 6**, show a population of cells with high acceptor to donor fluorescence ratio, and gated for collection. The bright oval-shaped cluster to the lower-right represents the bulk of data points, which are comprised of picodroplets containing low- or non-producing cells as well as empty picodroplets. The gating polygon is user-customizable for added flexibility so that the highest-value cells can be targeted for collection to microplates.

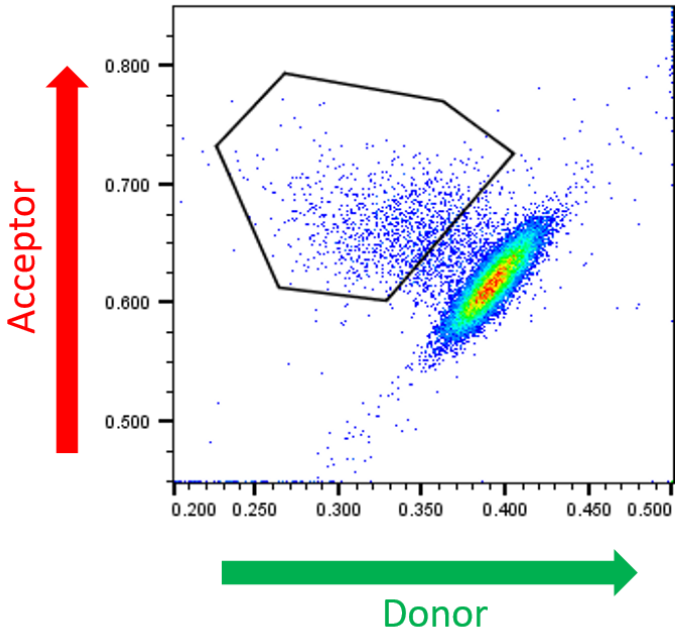

Figure 6: Cyto-Mine® Scatter Plot of FRET signal generated from picodroplet-encapsulated CHO cells incubated with Cyto-Mine® human IgG-specific AOF detection reagent.

Conclusions

These data show the power of Cyto-Mine® for screening large cell populations to find, sort and isolate high-value clones. The assay is quantitative in the range of immunoglobulin normally produced by standard biopharmaceutical production cell types. With its picodroplet-based IgG secretion assay, Cyto-Mine® offers the following major benefits:

Simplicity:

Seamless, one-step, automated process eliminating the need for multiple instruments

Quality:

Unique ability to mine and isolate rare, single, viable, high-producing cells

Sterility:

End-to-end sterile, disposable and AOF process

Speed and Efficiency:

Allows multiple projects to be run in parallel and for users to free up time for other activities

Miniaturisation:

Enables pL-level high-throughput screening with minimal use of assay reagents

Traceability:

Storable images proving single cell status at the point of dispensing provides the necessary documented evidence of monoclonality

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