Sphere Fluidics

Cyto-Mine®

Application Note 02

High-throughput method for mining B cell repertoires and hybridoma fusions to find rare cells secreting antigen-specific antibodies

This Application Note describes how Cyto-Mine® screens complex populations of primary B cells and hybridomas using a miniaturised, high-throughput, homogeneous secretion assay to analyse and identify antigen-specific clones for subsequent sorting and collection to microplates.

Introduction

Antibody-derived biologics have become a major class of modern medicine, particularly in the fight against cancer and autoimmune diseases. Highly efficacious immunoglobulin-based drugs have been developed naturally via the antibody-producing B cells of the mammalian immune system, although finding rare cells with the right characteristics has always been challenging. In the original protocol, mouse B cells needed to be immortalized through hybridoma generation. However, recent developments in single cell gene sequencing and recombinant immunoglobulin expression have enabled direct analysis of native B cells, thus allowing much more of the immune system diversity to be interrogated. This has opened up the potential to deep-mine the repertoire of any mammalian antibody-producing cell type.

Currently, semi-automated technologies such as cell sorting, colony picking and cell-in-well imagers are used in tandem to screen and isolate rare antigen-specific cells. The Cyto-Mine® Single Cell Analysis and Monoclonality Assurance System is the first fully integrated platform designed specifically for biopharma that screens millions of primary B cells or hybridomas for secreted immunoglobulin, identifies and sorts the antigen-specific candidates, and then gently dispenses them to 96- or 384-well microplates with visual proof of cell number (**Figure 1**).

This study shows the method by which the Cyto-Mine® antigen-specific assay analyses heterogeneous B cell and hybridoma populations to identify target-specific variants in a high-throughput manner.

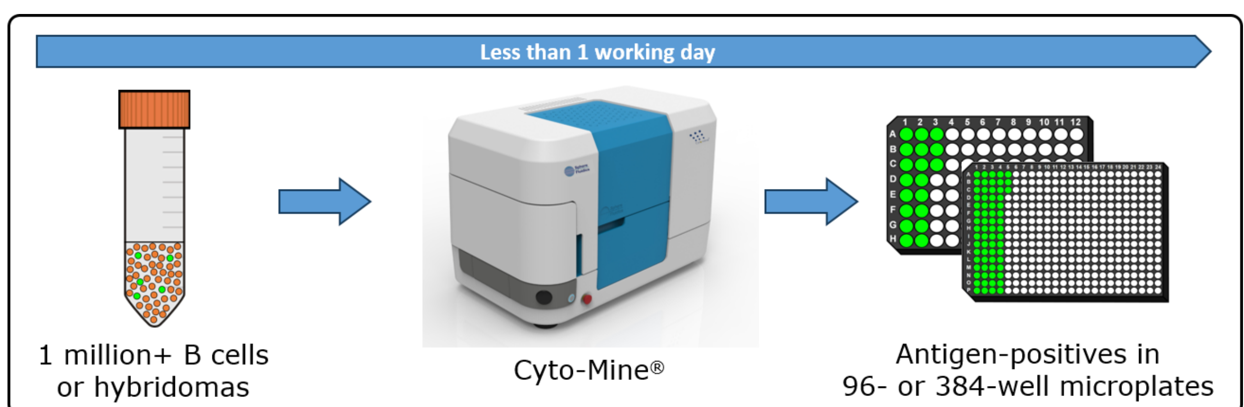

Figure 1. Cyto-Mine® technology finds and isolates antigen-specific cells from complex cell populations.

Methods

The B cell or hybridoma cell population of interest in its preferred culture medium is supplemented with the appropriate antigen-based detection reagents. The mix is then pipetted into a Cyto-Cartridge®, and the Cyto-Cartridge® loaded to Cyto-Mine®. Cyto-Mine® initiates the process by encapsulating the cells into 300pL picodroplets. The number of cells per picodroplet can be varied according to user requirement, e.g. 1 cell per picodroplet if single-cell cloning is essential, or more than 1 cell per picodroplet if the population size exceeds 1 million cells, with the latter leading to collection of enriched mini-pools of cells rather than single cells. Cyto-Mine® then incubates the cells *in situ* to allow secretion of immunoglobulin into the picodroplets. The miniaturized format enables an ultrasensitive, rapid assay of typically 0.5 to 2 hours depending on the production rate of the cell population. Antigen-positive hits are subsequently sorted for picodroplet by picodroplet collection. The integrated stages of the Cyto-Mine® antigen-specific assay process are summarized in **Figure 2**.

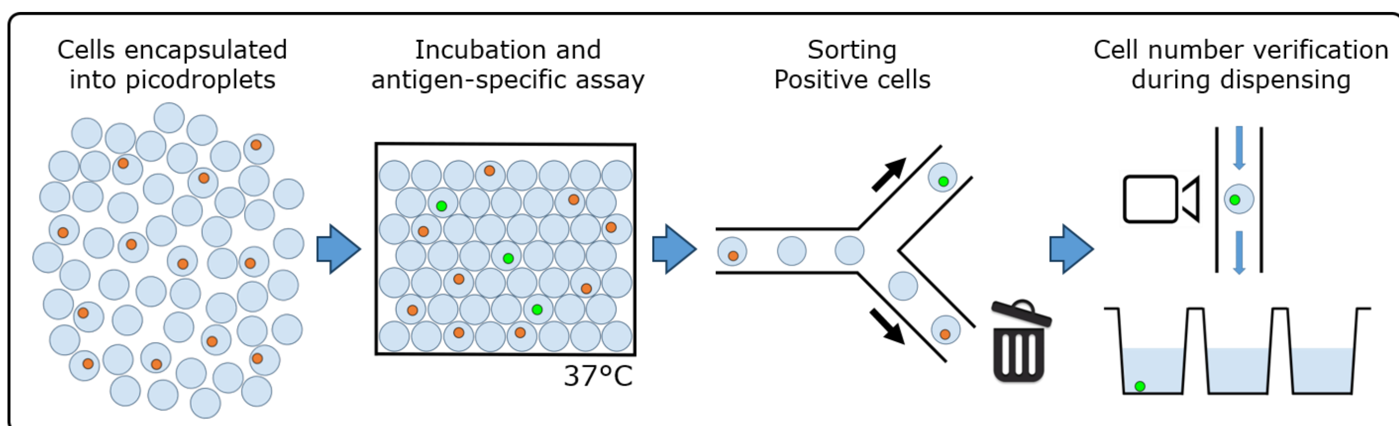

Figure 2. The Cyto-Mine® workflow integrates the screening, sorting, isolation and verification of antigen-specific clones into a fully automated process.

Cyto-Mine® antigen-specific assay

A key enabling component of Cyto-Mine® is its ability to analyse the secreted immunoglobulins of millions of cells for antigen specificity while maintaining the cells in a highly viable state with no prior modification. The method by which positives are reported is presented in **Figure 3**. The detection probe pair (fluorescently-conjugated antigen and an immunoglobulin-specific acceptor probe) bind to the secreted antigen-specific immunoglobulin inducing a FRET-mediated shift in fluorescence. Cyto-Mine® then measures the fluorescent signal generated and converts it to a quantitative output. The choice of acceptor probe permits simultaneous screening for antigen specificity and immunoglobulin isotype

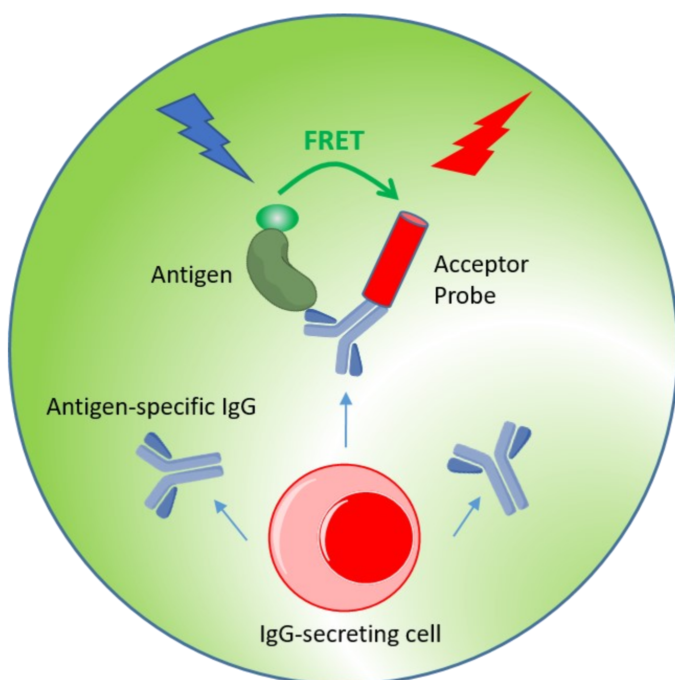

Figure 3: The Cyto-Mine® picodroplet-based antigen-specific assay. This model shows the standard assay to screen for antigen-specific IgG. Antigen-specific IgG secreted from the encapsulated cell during incubation is recognised by both the antigen and the IgG-specific acceptor probe forming a 3-body FRET complex that induces a fluorescent signal.

Results

Antigen-specific assay titration curve

To confirm that the Cyto-Mine® antigen-specific assay is quantitative, titration experiments were undertaken using human TNF-alpha as a model antigen. Cell culture medium was spiked with 5 different concentrations of anti-human TNF-alpha IgG up to 20µg/mL (equivalent to 133nM) and then encapsulated into picodroplets containing TNF-alpha and IgG-specific detection probes. The 5 picodroplet populations were then pooled and put through the Cyto-Mine® process to measure antigen specificity. The results, presented in **Figure 4**, show how the different titers were resolved as discrete populations. The titration curve shown in **Figure 5** demonstrates that the assay is specific and quantitative.

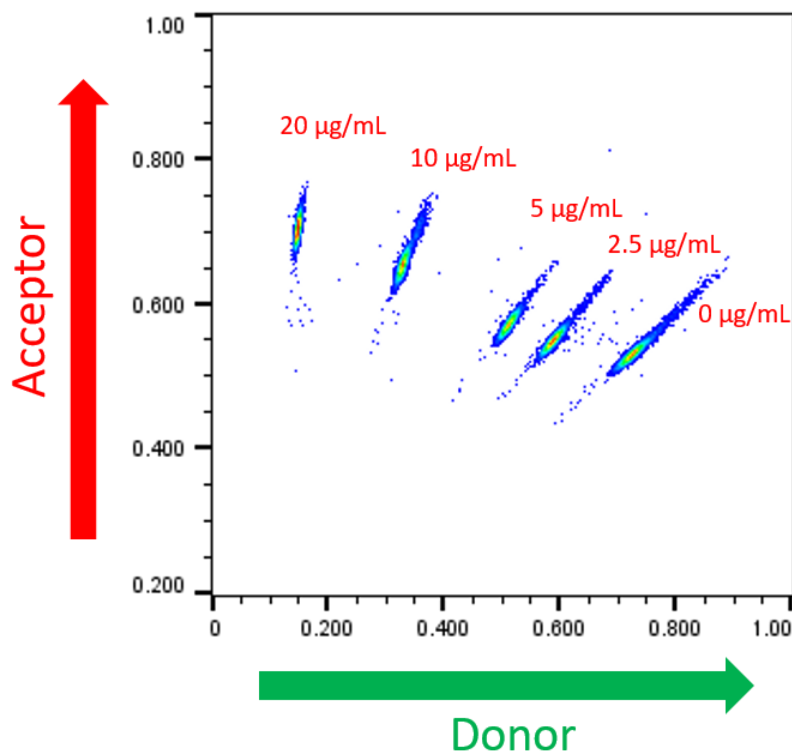

Figure 4: Cyto-Mine® Scatter Plot. Large numbers of individual picodroplets were loaded with the indicated concentrations of anti-human TNF-alpha IgG and then resolved using the Cyto-Mine® antigen-specific assay and analysis.

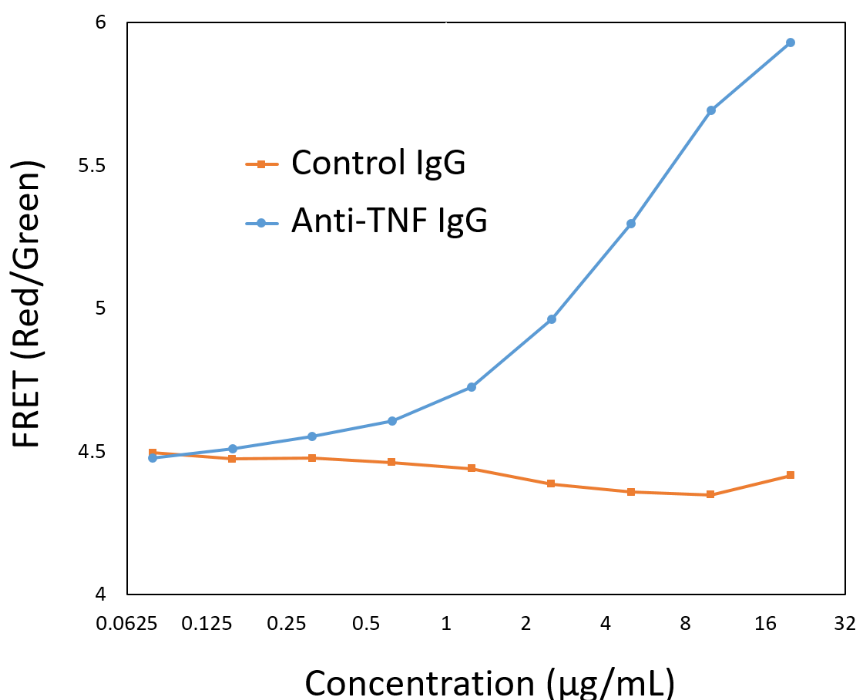

Figure 5: Representative titration curve generated using the Cyto-Mine® antigen-specific assay. In this example, the control (irrelevant) IgG confirms specificity of the assay for human TNF-alpha.

Screening hybridomas for antigen-specific clones

A hybridoma fusion generated from a mouse immunized with human TNF-alpha was run on Cyto-Mine® to find TNF-alpha specific clones. The cell population was mixed with fluorescently-conjugated human TNF-alpha and acceptor probe against mouse IgG-Fc, and run through the Cyto-Mine® antigen-specific assay workflow. The results, presented in **Figure 6**, show a population of cells with high acceptor to donor fluorescence ratio, which have been gated for collection. The bright oval-shaped cluster in the lower-right corner represents the massive bulk of negative data points, which are comprised of empty picodroplets, and picodroplets containing either non-antigen-specific cells or non-producers. In this experiment, the gating polygon has been set to sort all antigen-positive clones but is fully customisable to suit experimental needs.

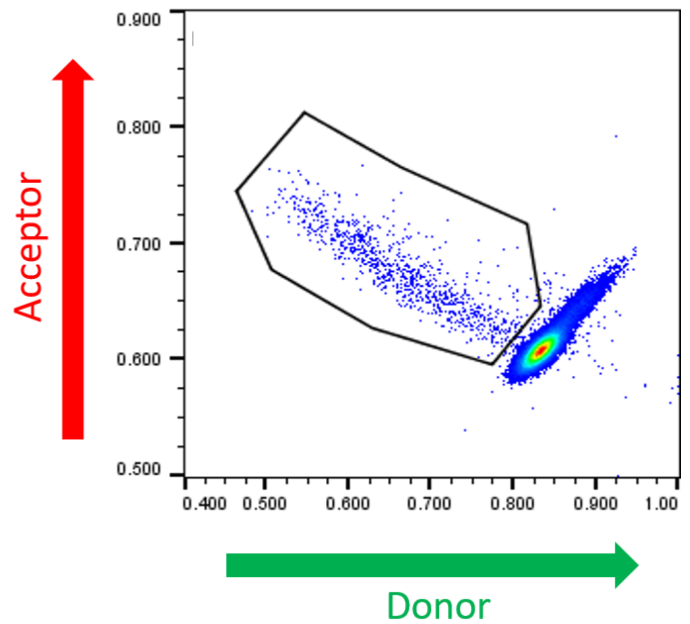

Figure 6: Cyto-Mine® Scatter Plot of FRET signal generated from hybridomas encapsulated in picodroplets and screened for secretion of human TNF-alpha-specific IgG.

Conclusions

Data presented here shows the value of Cyto-Mine® to find and sort rare antigen-specific cells from large populations of B cells and hybridomas. With its picodroplet-based antigen-specific assay, Cyto-Mine® offers the following major benefits:

Simplicity:
A seamless, one-step, automated process eliminating the need for multiple instruments

Quality:
Deeper immune sampling to find and isolate rare antigen-specific cells based on actual secretion

Speed and Efficiency:
Shortens the Discovery process timeline and enables multiple projects to be run in parallel with no downtime between runs

Cell Compatibility:
Picodroplet encapsulation ensures high viability of selected clones (90–100%) and compatibility with a broad range of human and animal cell types

Sterility:
Benchtop system compatible for use in Class II biosafety cabinets, with end-to-end sterility and disposable consumables

This work is partly funded by the UK Government Advanced Manufacturing Supply Chain Initiative (AMSCI) as part of the BioStreamline Project which exists to develop new approaches for the more efficient development and manufacture of next generation biologics.
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