Rapid B Cell Screening in Cyto-Mine®

Introduction

High-throughput technologies for single B cell screening provide many benefits in comparison to traditional hybridoma technology during lead discovery in the development of therapeutic antibodies.

Introduced by Köhler and Milstein in 1975, hybridoma technology has produced the highest success rate for FDA-approved therapeutic antibodies [1]. However, one of the most significant limitations of this technology is the paucity of hit candidates within a large cell population. Only a fraction of the total B cell population from an immunized animal are taken forward from the fusion reaction, of which a small percentage will be secreting antibodies. From those, a minute number will be secreting antibodies to the target of interest. A lot of these key antibody-secreting cells are lost during cell processing and fusion, so consequently, risking missing valuable potent hits. The development of technology that can provide screening of the whole B cell population is paramount to ensure that key target-specific antibody-secreting cells are not missed.

Cyto-Mine® provides a platform that takes the pain out of finding those rare cells from large B cell populations to facilitate the progression and acceleration of antibody discovery.

This application note illustrates how the novel picodroplet technology of Cyto-Mine® offers a robust, high throughput platform for screening whole B cell repertoires for antigen-specific antibody-secreting B cells in just two days while supporting cell viability.

Cyto-Mine® can screen, select and dispense antibody-secreting cells with antigen specificity by encapsulating up to 40 million cells in one run, assaying for the target antigen, selecting antigen-specific antibody-secreting cells, and dispensing as a pooled population or as single cells into 96- or 384-well microtiter plates.

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Aims and objectives

- Demonstrate the application of Cyto-Mine® to robustly screen whole B cell repertoires for antigen-specific antibody secreting cells.
- Showcase the ability of Cyto-Mine® to rapidly screen multiple cells/picodroplets (40 million cells) or single cells/ picodroplet (1-2 million cells) in one day.
- Demonstrate assay flexibility of Cyto-Mine® (antigen-specific assay or more general IgG assay).



Method

Sample Preparation

Frozen lymphocytes from an immunized mouse were thawed and washed with fresh culture medium by centrifuging at 200 g for 5 minutes and resuspending the cell pellet in a suitable fresh culture medium at room temperature. Centrifugation was then repeated, and the cell pellet resuspended in Encapsulation Medium, as shown in Table 1. The cell suspension was filtered through a 30 µm cell strainer to remove any cell clumps or debris. Then the cells were counted and diluted to the desired working concentration using Encapsulation Medium. To 1 mL of the cell sample, either IgG or antigen-specific FRET assay reagents were added. The sample was then pipetted into a Cyto- Cartridge® and loaded into the Cyto-Mine®.

Encapsulation Medium Component	% (v/v)
Preferred culture/cloning medium (fresh)	74%
OptiPrep™ Density Gradient Medium (Iodixanol 60% w/v) 16%	16%
Pluronic™ F-68 (10%)	10%

Table 1.

Cyto-Mine® Workflow

Cyto-Mine® first encapsulates cells within 450 pL picodroplets of preferred culture medium (Figure 1). Droplets occupancy is determined by using cells at a dilution level that optimizes the number of picodroplets containing the required number of cells per droplet [2]. After encapsulation, the cells are incubated

to reach detectable level of secreted antibodies and assayed to identify secreted antibodies using previously developed assay. For this study, an IgG and an antigen-specific assay were used on different days over a two-day screen. Cyto-Mine® selectively sorts the "hits" and then during subsequent dispensing into individual microtiter plate wells, images the picodroplets for assurance of clonality.

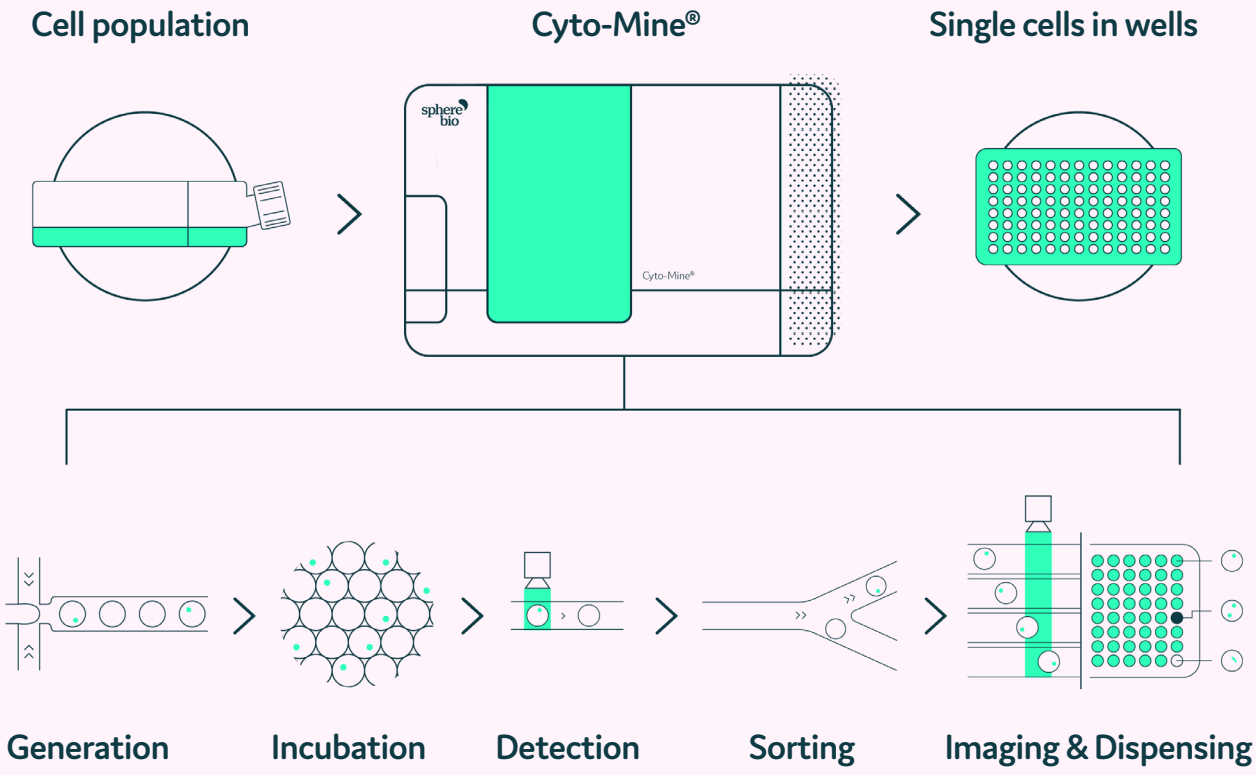

Figure 1. A Cyto-Mine® workflow. Integration of single cell screening, sorting, isolation, and verification using a fully integrated microfluidic process.

In this study, an initial assay validation step was carried out to ensure that the developed FRET assay probes were able to selectively detect IgG and antigen-specific antibodies secreted from B cells isolated from mouse lymphocytes.

After assay validation, a two-step screening approach was used to screen 40 million cells to isolate B cells secreting antigen-specific antibodies to the target antigen. The first round of screening demonstrated the capacity of Cyto-Mine® to analyze a whole B cell repertoire of ~40 million B cells for enrichment of picodroplets containing cells secreting IgG antibodies. Then cells were recovered and the next day this first screening was followed by a second round of screening to isolate single B cells secreting antigen-specific antibodies.

For this study, we utilized the flexibility of Cyto- Mine® to enable encapsulation of pools of cells within picodroplets for the first round of screening (Figure 1B) and single cell encapsulation for the second round of screening (Figure 1A). Based on Poisson distribution statistics, the cell concentration within the sample dictates the number of cells encapsulated per picodroplet (Table 1) [2].

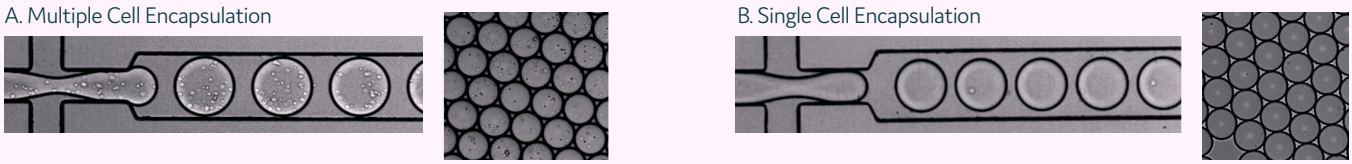

Figure 1. These images show the encapsulation of single cells or multiple cells per picodroplet. A) A large population of cells (greater than 1 million) were diluted to a concentration of $1x10^8$ cells/mL in medium resulting in multiple cells per picodroplet. B) Cells were diluted to a concentration of $1x10^6$ cells/mL to obtain population of mostly 1 cell per occupied picodroplet.

Cyto-Mine® Assays

One of the key enabling components of Cyto-Mine® is its ability to measure the specific secretion of every single cell. The starting cell population requires no prior modification, it is mixed with the appropriate detection probe pairs before loading on to Cyto-Mine®. The detection probes bind to the secreted antibodies of interest inducing a FRET-mediated fluorescent signal, illustrated in Figures 2 and 3.

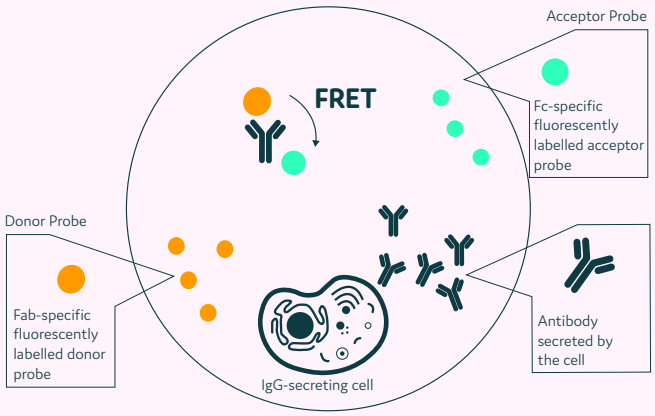

Figure 2. Positive events readout using the Cyto- Mine® picodroplet-based FRET IgG secretion assay. This model shows the standard assay to screen for IgG secreting cells. A customized pair of IgG-specific fluorescent probes are trapped within each picodroplet during encapsulation. IgG secreted from the encapsulated cell is recognized by the detection probe pair forming a 3-body FRET complex that induces a shift in fluorescent signal.

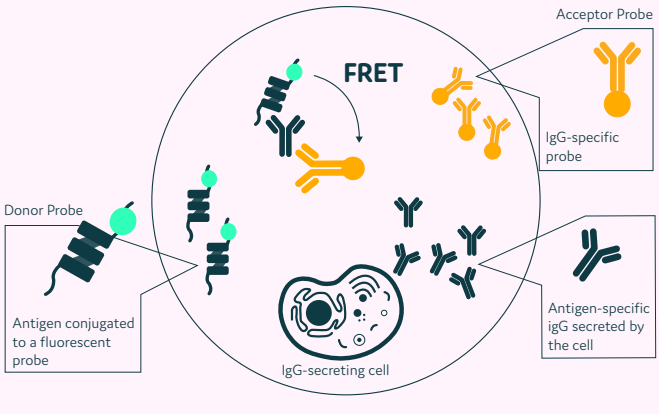

Figure 3. Positive events readout using Cyto-Mine®picodroplet-based FRET antigen-specific assay. A customized pair composed of fluorescent antigen and fluorescent IgG probe are trapped within each picodroplet during encapsulation. Only Antigenspecific IgG secreted from the encapsulated cell is recognized by the detection probe pair forming a 3-body FRET complex that induces a shift in fluorescent signal.

Results

Assay validation

A sample of B cells isolated from mouse lymph nodes was analyzed using Cyto-Mine® first to identify picodroplets containing IgG-secreting cells. Second to determine if the secreted antibodies could be detected by our antigen-specific detection probe created for the target antigen. Figure 4 shows the results from the two separate assays, where the isolated B cells were deteted to secret either general IgG (left panel) or the target antigen-specific antibodies (right panel).

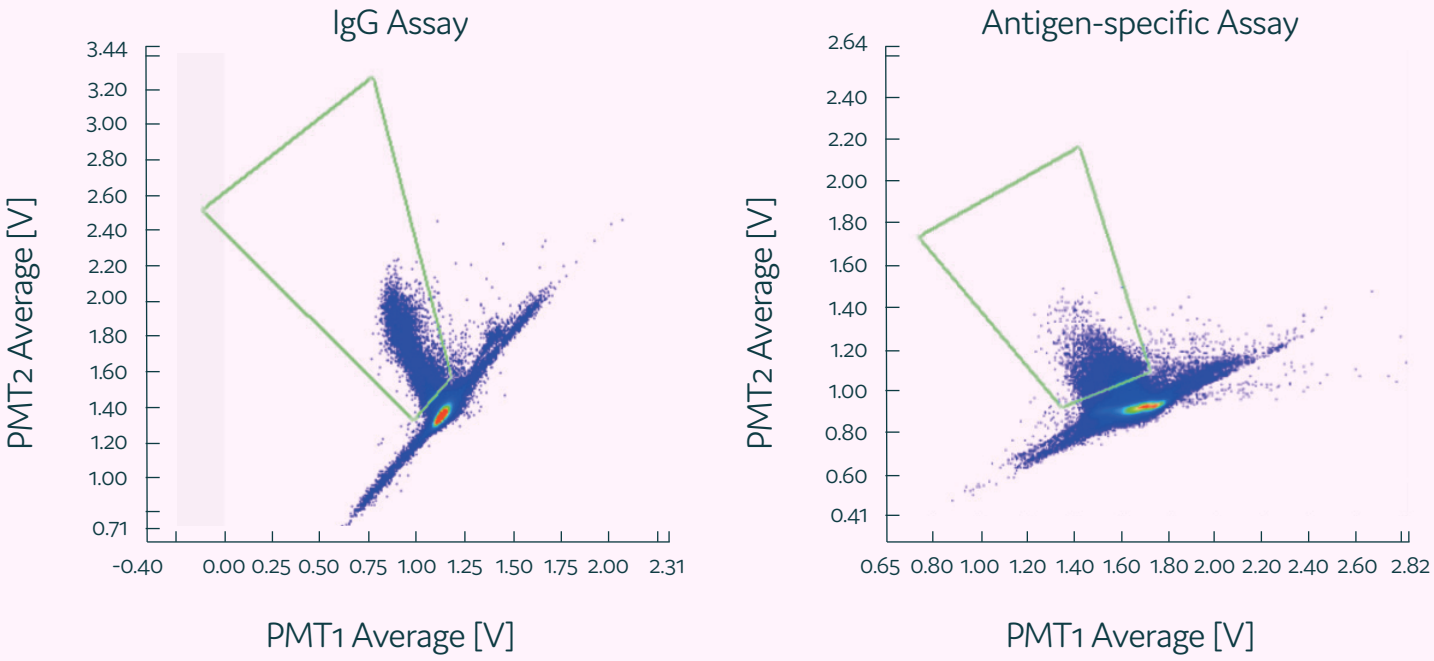

Figure 4. Each of these scatter plots shows representative example of a positive population obtained with different assays indicating the binding of the assay probes to the secreted antibodies.

The efficient screen of a whole B cell repertoire

Round 1 screening

To detect all IgG secreting cells, a first screening round was conducted on the repertoire of 39 million cells using IgG FRET assay. Multiple cells were encapsulated in picodroplets and positive picodroplets were sorted and consequentially dispensed into one well of 6-well plate according to the pooled picodroplet dispensing protocol, thus resulting in population enriched in IgG-secreting cells. Given the fact that encapsulation resulted in occupancy of all the picodroplets with multiple cells and that the rate of positive events during enrichment run was 0.22%, only small proportion of cells were actively secreting IgG (Figure 5).

Viability of input cell sample	75.4%
No. cells screened	$3.9x10^7$
No. cells per picodroplet (λ)	32.7
Measured positive rate for current experiment	0.22%
Viability of cells collected from positive hits	61.20%

Figure 5. Multiple B cell screening.

Cell viability was recorded throughout the whole workflow and as shown in Figures 5 and 6, Cyto- Mine® preserves the viability of Primary B cells during analysis and incubation, demonstrating compatibility of this platform with two-round screening of primary cells.

Round 2 screening

After overnight culture, a second screening round was completed using an antigen-specific FRET assay, to detect cells secreting antigen-specific antibodies. Now single cells were individually encapsulated in picodroplets, and positives droplets were dispensed in wells containing lysis buffer (Figure 7). As shown in Figure 8, similar experiments were successfully reproduced 3 times, indicating feasibility of this approach as a routine approach.

Percentage of viable cells	
Cells viability prior to Cyto-Mine analysis	75%
Cells viability post Cyto-Mine analysis 61%	61%
Cells viability after overnight culture 65%	65%

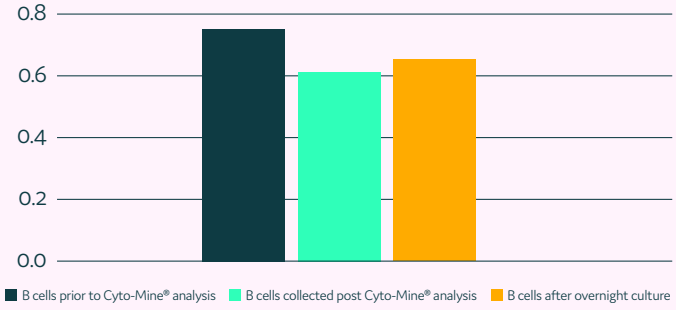

Figure 6. Primary B cell viability after Cyto-Mine® runs.

Viability of input cell sample	65.4%
No. cells screened	421,300
No. cells per picodroplet (λ)	0.36
Measured positive rate for current experiment	0.06%

Figure 7. Single B cell screening.

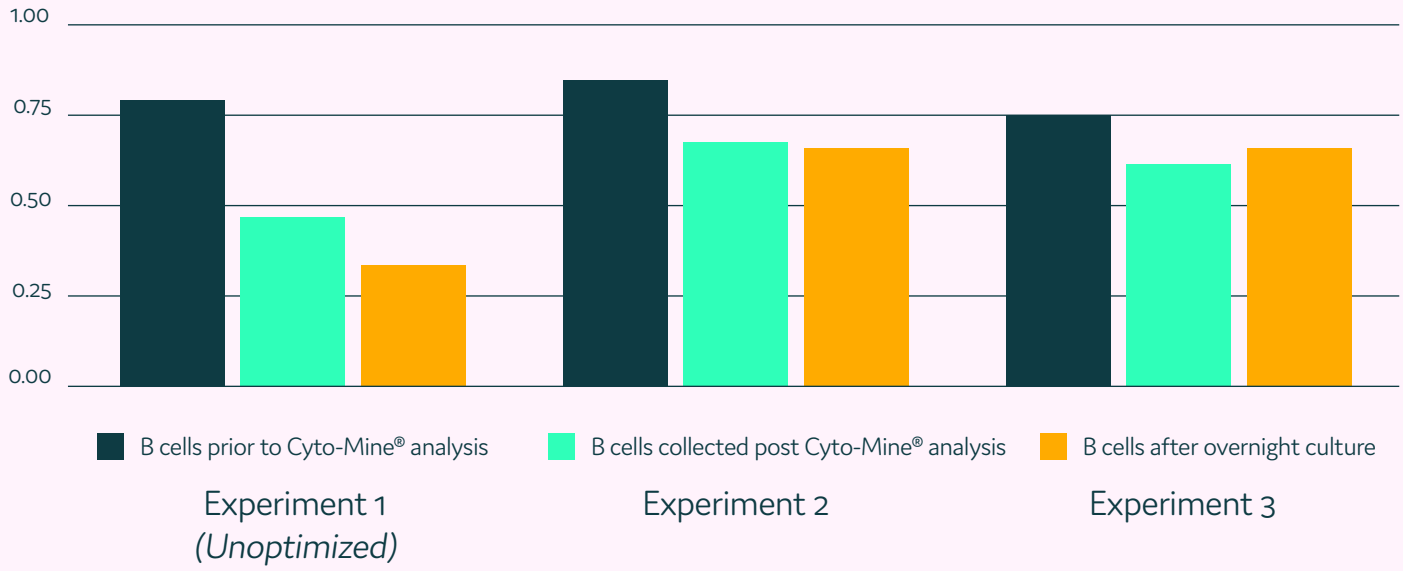

Figure 8. Repeated single B cell screening experiments.

Conclusions

These data show the power of Cyto-Mine® for efficiently screening whole B cell repertoires to both identify antibody-secreting cells and isolate rare cells secreting antigen-specific antibodies. The two-step process first used a bulk assay to enrich for IgG secretion cells, then used an antigen detection assay to probe for antigen-specific antibodies and isolate the rare cells secreting them, all in only two days. Importantly, throughout the process, the cell viability of the B cells was maintained.

Speed:

The high-throughput method to accelerate the discovery of antigen-specific antibodies in the discovery and development of monoclonal antibodies

Simplicity:

The continuous, integrated and automated process eliminates the need for multiple instruments

Efficiency:

Miniaturized screening offers the deep interrogation of large populations of antibody-secreting cells (total B cell repertoires) to overcome the low efficiency in hybridoma technology

Cell Compatibility:

Picodroplet encapsulation ensures good viability of the B cells throughout the isolation process

References

1. Lu, Z.-J.et al., 2012. Frontier of therapeutic antibody discovery. World Journal of Biological Chemistry, pp. 187-196.
2. Josephides, D. et al. (2020). Cyto-Mine: An Integrated, Picodroplet System for High-Throughput Single-Cell Analysis, Sorting, Dispensing, and Monoclonality Assurance. SLAS TECHNOLOGY: Translating Life Sciences Innovation. https://doi.org/10.1177/2472630319892571

Notes

Notes

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