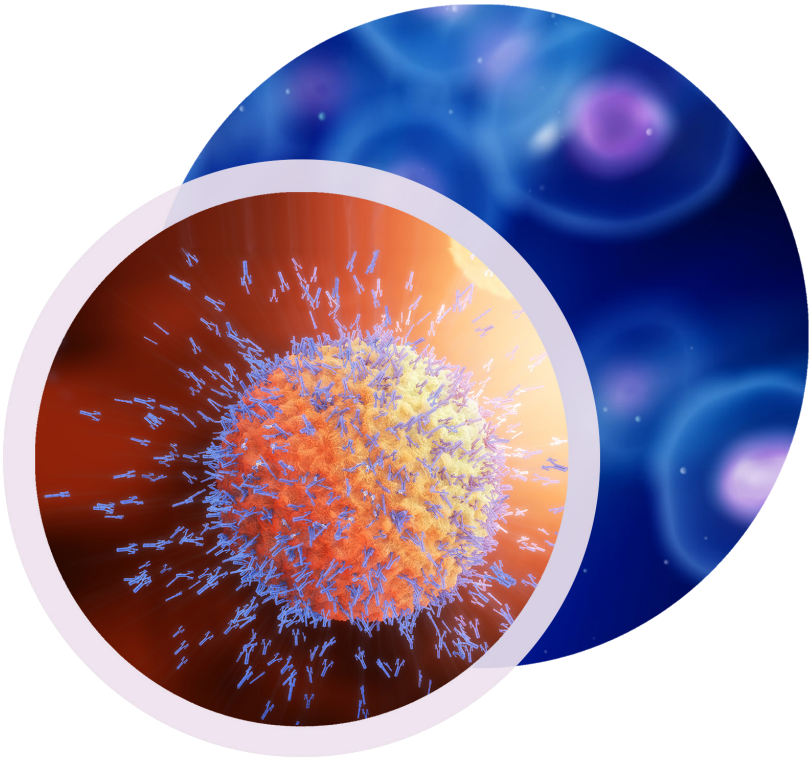


Accelerating Antibody Discovery with Single B Cell Screening

As researchers race to identify new therapeutics, streamlined antibody discovery processes have never been more crucial, and the need for speed and efficiency is paramount. The discovery of therapeutic antibodies relies on the deep interrogation of whole B cell repertoires or hybridoma populations. Yet, screening efforts are time-consuming and costly using traditional methods. This creates major bottlenecks in finding lead candidates for progression to antibody optimization and clinical candidate selection.

In this eBook note, we describe a robust and rapid single B cell screening method to identify antibodies of desired antigenic specificity without the laborious need for traditional hybridoma screening. Sphere Fluidics' Cyto-Mine® Single Cell Analysis System, harnesses proprietary picodroplet-based technology to capture and screen antibodies directly from whole B cell repertoires, preserving cell viabilities and dramatically improving efficiencies. Using this method, researchers can identify antibody-secreting cells and isolate rare cells secreting antigen-specific antibodies in just two days, significantly accelerating the time-to-market for therapeutics.

Sphere Fluidics

Challenges In The Antibody Discovery Process

Antibody discovery typically starts by either creating hybridomas by fusing a B cell and a myeloma cell populations, or by utilizing technologies such as phage display. All these processes require several iterative screening runs to interrogate antibody libraries and identify leads. The leads are then characterized, optimized, and taken to pre-clinical development.

Despite technological and methodological advances, there are several limitations still to be overcome. First, hybridoma generation is time-consuming, costly, and inefficient, as this approach may result in missing the rare antigen-binding antibody with the desired characteristics. During hybridoma generation, many B cells die-off in culture and do not undergo successful fusion and, consequently, the key antibody-secreting cells may be lost. Second, traditional approaches only interrogate a small subset of the cell repertoire. In the hybridoma scenario, a large part of the B cell repertoire is lost during hybridoma generation, so only a fraction of the total population is screened, and in turn, the most potent candidate molecules may not be identified. Considering the population of target cells can be as low as 0.001% of the original ~40 million cells harvested, this is a significant challenge to address. 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.

High-throughput screening (HTS) remains the most successful lead generation strategy; when applied to the development of therapeutic antibodies, it reduces the time required to identify and isolate the rare antigen-specific, antibody-secreting cells from a population of millions. This is typically done by screening the purified B cells directly with flow cytometry, bypassing both hybridoma fusion and phage display. Flow cytometry has the advantage of being very high-throughput, and antibodies secreted by B cells can potentially be screened using cold capture, a technique used to prevent the full secretion of antibodies by trapping them at the cell surface. However, this is a representation rather than direct measurement of the antibody secretion profile by a single cell. Additionally, flow cytometry can be harsh on the cells, especially primary cells, and may alter cell function and viability. Alternative screening methods include ELISA and Elispot; these techniques however often need to be executed manually and, consequently, it becomes too costly and time-consuming to analyze large populations.

After multiple rounds of screening and selection, the positive cells must be sub-cloned into monoclonal populations (lead panels) by employing semi-automated methods like cell-in-well imagers and cell sorting; this multi-step approach adds even more complexity and hands on-time, slowing down the process even more (1).

Achieving Improved Process Efficiency With Microfluidics

The rise of microfluidics and, in particular, droplet microfluidics, has further advanced the field of HTS. Microfluidic systems conduct complex multi-step assays with high reliability, cost-efficiency and throughput in a picolitre volume water-in-oil emulsion droplet (picodroplet) format. Picodroplets bring the advantages of miniaturization and automation, thus helping to improve laboratory productivity by facilitating rapid, high-throughput research to interrogate bigger repertoires and find more functional properties (2).

Leveraging picodroplet-based technology, Cyto-Mine® offers a robust, high throughput platform for screening whole B cell repertoires for antigen-specific antibody-secreting B cells. The automated platform can screen, select and dispense antibody-secreting cells with antigen specificity in just two days. The two-step screening approach involves using a bulk assay to enrich for B cells that present the antibody of interest, then using an antigen detection assay to screen for antigen-specific antibodies. The isolated antigen-specific antibody-secreting cells are then dispensed as pooled population or as single cells into 96- or 384-well microtiter plates for further analysis.

Encapsulation in picodroplets ensures that concentration of the molecules secreted by the cell increases extremely quickly, and protects single cells from shear stress, thereby overcoming some of the major barriers of flow cytometry and fluorescence-activated cell sorting. In this two-step process, the flexibility of Cyto-Mine® enables the encapsulation of pools of cells within picodroplets for the first round of screening (illustrated in Figure 1B) and single cell encapsulation for the second round of screening (illustrated in Figure 1A)(3).

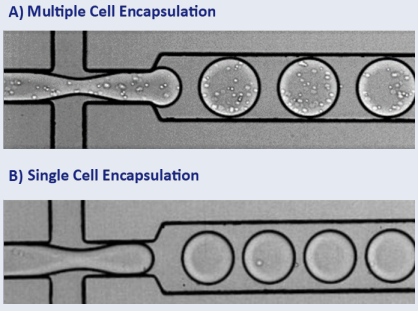


Figure 1. These images show the encapsulation of single cells or multiple cells per picodroplet. A) A large population of cells (greater than 1 million) were diluted to a concentration of $1x10^8$ cells/mL in medium resulting in multiple cells per picodroplet. B) Cells were diluted to a concentration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

Overall, Cyto-Mine® addresses the major challenges faced in the antibody discovery workflow which are:

- **Flexibility** - adaptable assay design for specific needs
- **Measurement** - semi-quantitative assays of antibody secretion
- **Sensitivity & specificity** - detect antibodies of interest
- **Efficiency** - screen the entire cell population
- **Viability** - maintain high levels of cell viability
- **Speed** - reduce total workflow timelines

Assay Flexibility

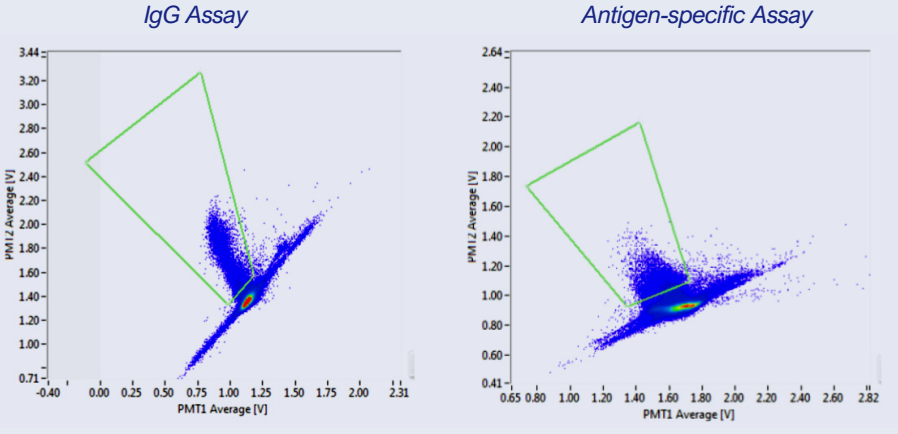
Antigen-specific assays developed by Sphere Fluidics enable the detection of specific targets. We also offer flexible assay design, including the ability to develop tailored assays to suit your specific application needs. Assays may include antigen-specific assays for B cell mining or hybridoma screening if needed.

Antibody Measurement

Picodroplet-based technologies offer a highly-sensitive method to assay and select antigen-specific antibody-secreting cells in ways that conventional methods cannot.

To demonstrate that the Cyto-Mine® antigen-specific assay is semi-quantitative, experiments were carried out on a sample of B cells isolated from mouse lymph nodes. 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 2 shows the results from the two separate assays, where the isolated B cells were detected to secret either general IgG (left panel) or the target antigen-specific antibodies (right panel).

Figure 2. 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



Sensitivity & Specificity

Leveraging our proprietary picodroplet technology and assay design flexibility, Cyto-Mine® provides a powerful tool to find, sort and select rare antigen-specific cells from large populations of antibody-secreting cells, as illustrated in Figure 3 and 4.

Figure 3. Round 1 Screening
To detect all IgG secreting cells, a first screening round was conducted on arepertoire 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 a 96-well plate.

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

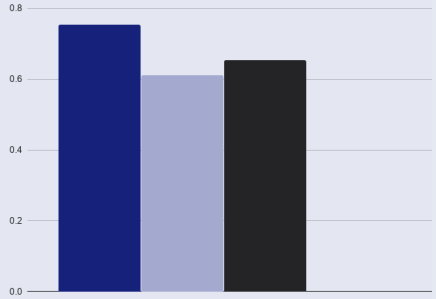
Figure 4. Round 2 Screening
After overnight culture, a second screening round was completed using an antigen-specific FRET assay, to detect cells secreting antigen-specific antibodies. Now single cells were individually encapsulated in picodroplets, and positive picodroplets were dispensed in wells containing lysis buffer.

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

Cell Compatibility

Picodroplets provide a uniquely protective environment to support cell viability and integrity during incubation, and shield cells against shear stress as they flow through the microfluidic channels. The biocompatible surfactant generates picodroplets that are stable for many days and promotes high rates of gas exchange which maximises cell viability. Figure 5 provides an example of how 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.

Figure 5. Primary B cell viability after Cyto-Mine® runs

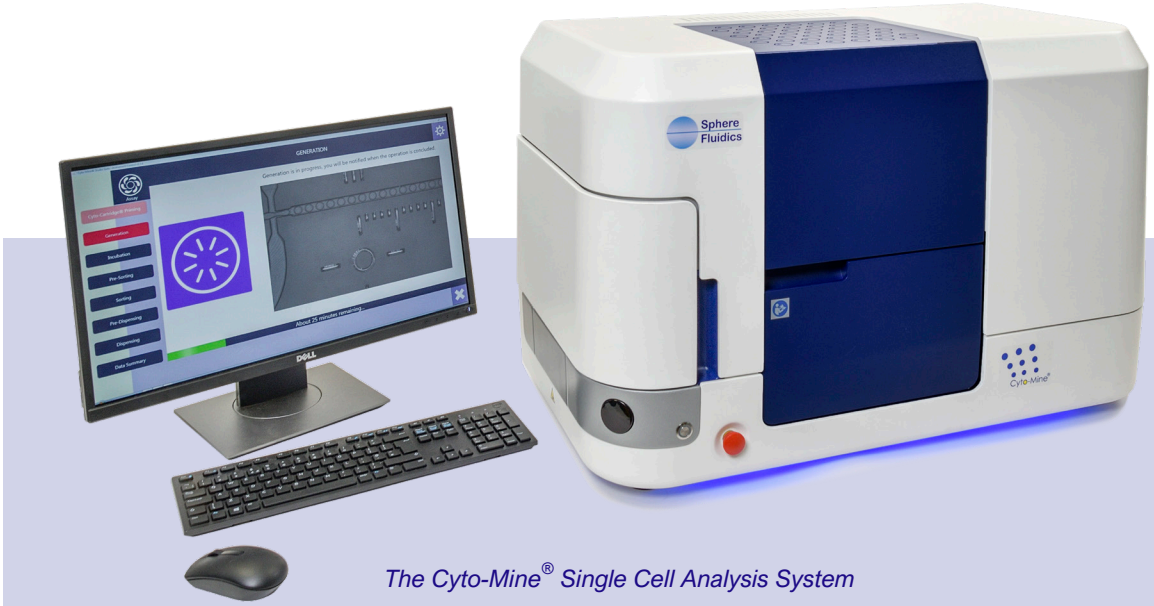


Percentage of viable cells	
Cells viability prior to Cyto-Mine analysis	75%
Cells viability post Cyto-Mine analysis	61%
Cells viability after overnight culture	65%

B cells prior to Cyto-Mine analysis
B cells collected post Cyto-Mine analysis
B cells after overnight culture

Significant Time Savings

Cyto-Mine® provides a platform that takes the difficulty out of finding those rare cells from large B cell populations to facilitate the progression and acceleration of antibody discovery. In just two days, 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 into 96- or 384-well microtiter plates.



The Cyto-Mine® Single Cell Analysis System

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