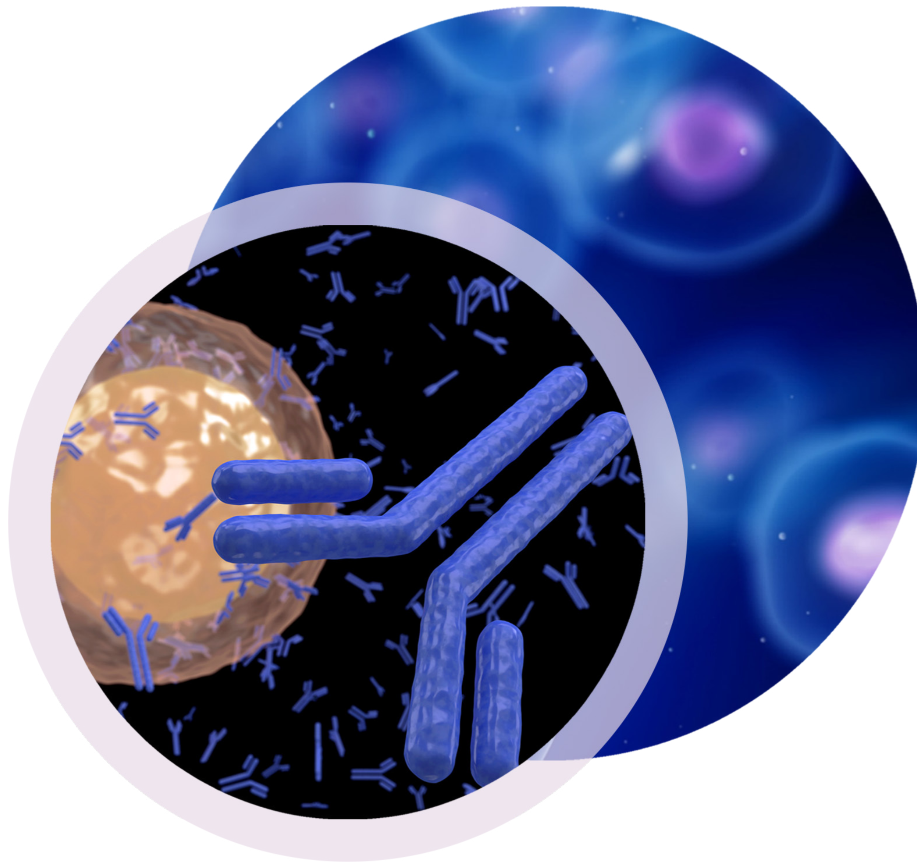


Accelerating Hybridoma-Based Antibody Discovery with Cyto-Mine®

The rise in monoclonal antibody based therapeutics over the past 20 years has been extraordinary with over 80 mAbs now approved for use. With antibody-based therapeutics entering clinical studies and being approved faster than ever before their success, and increasing competition, is creating a large demand on biopharmaceutical companies to reduce the time it takes to bring new biologics to market.

Traditional methods for screening large panels of cells used in antibody discovery during lead discovery are resource- and labor-intensive requiring many different items of equipment for each step such as single-cell analysis, sorting, imaging and dispensing into individual wells of microtiter plates. This creates major bottlenecks in finding lead candidates for progression to antibody optimization and clinical candidate selection.

Cyto-Mine® addresses the time-consuming, labor-intensive nature of screening large cell populations for rare antigen-specific, antibody-secreting cells during antibody discovery by streamlining the traditional multi-step process into one seamless, fully-integrated one-day workflow.

HYBRIDOMA-BASED ANTIBODY DISCOVERY

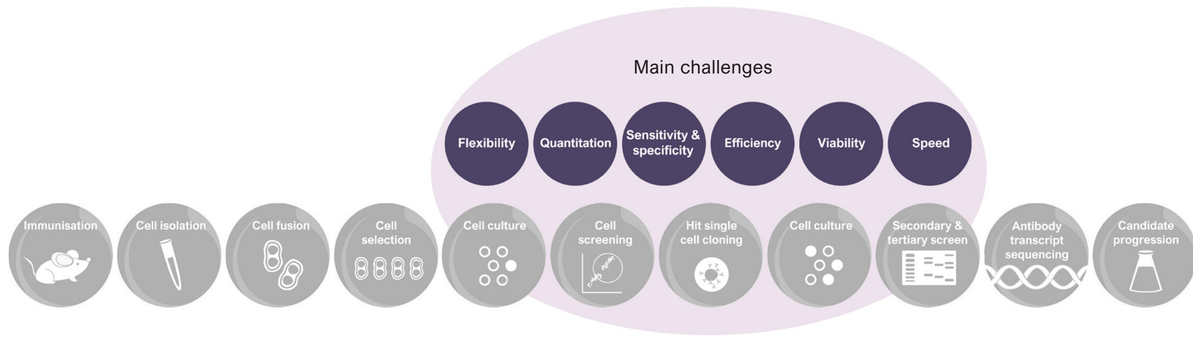


Figure 1. Traditional workflow in hybridoma screening

In antibody discovery, the generation of early candidates typically starts with a known, validated target where candidate antibodies need to be generated for 'Hit' selection. Traditionally, animals, whether they are regular or transgenic mice, rats or rabbits, are immunized to stimulate an immune response, after several weeks B-cells can be harvested and fused with myeloma cells to create hybridoma populations (Figure 1). Alternatively, the B-cells can be isolated directly but, whichever process is adopted, the cells producing the antibodies require extensive screening and characterization. This is where bottlenecks typically occur when using conventional screening methods such as flow cytometry and ELISA. Flow cytometry is a well-established, high throughput method for screening large cell populations, however, it comes with some significant downsides. The process of flow cytometry is harsh and can result in shear stress that can cause damage to any cell including those that are rare or valuable, therefore reducing cell viability and potential recovery. Additionally, flow cytometry cannot be used to readily measure cell secretion, flow cytometers offer cold capture secretion assays where the secreted molecules are retained at the cell surface using cell manipulation but any secreted molecules not captured diffuse into the extracellular environment, as such, flow cytometry does not provide an accurate representation of cell secretion.

Once the antibodies have been screened and characterized the cells then need to be subcloned into monoclonal populations. This can be done using semi-automated technologies such as cell printers and cell-in-well imagers, adding multiple instruments into the workflow, in turn making the whole process very time consuming and labor intensive.

HIGH-THROUGHPUT ANTIBODY DISCOVERY SCREENING

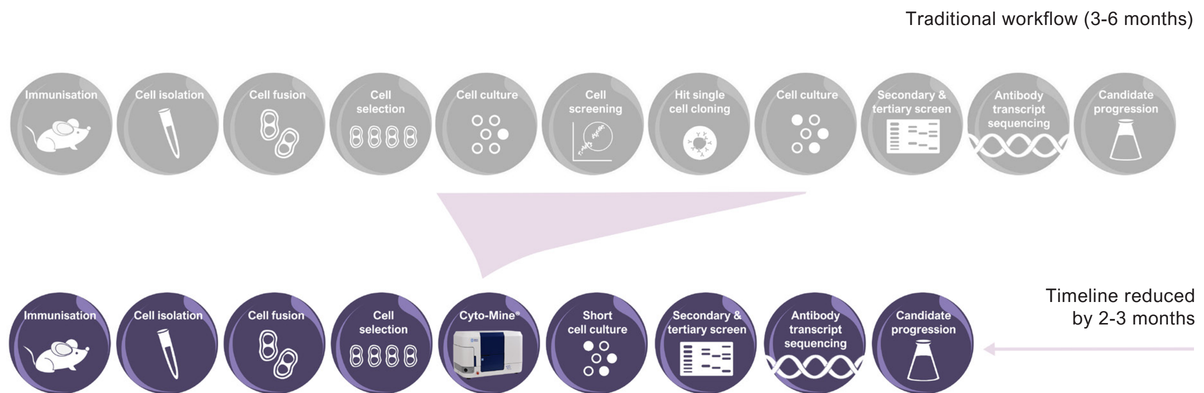
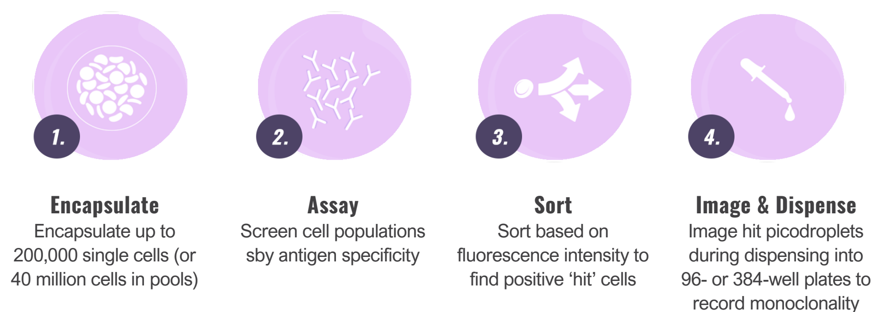


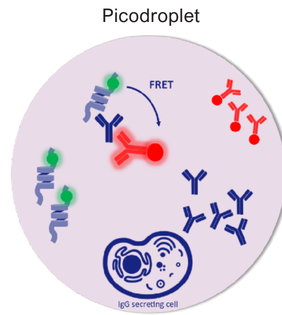
Figure 2. Accelerated workflow in hybridoma screening

Cyto-Mine® is a fully-integrated platform based on microfluidic picodroplet technology. The integrated platform combines cell isolation, assay, sorting, imaging and dispensing into one automated workflow to significantly reduce timelines for the discovery and development of antibody-based therapeutics (Figure 2).



In doing so, 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 hybridoma screening or B-cell mining.

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, titration experiments were carried out using human TNF-alpha as a model antigen. The results, presented in Figure 3, show how known concentrations of antigen were resolved as distinct populations. As a control, the samples were analysed alongside control IgG not expressing the antigen using a fluorescent plate reader (Figure 4). The titration curve shown in Figure 4 demonstrates that the antigen-specific assay probes only detected the human TNF-alpha population and not the control IgG, consequently, the assay is specific for the antigen of interest. Then in Figure 5, a hybridoma population secreting human TNF-alpha-specific IgG at unknown concentrations was analysed to demonstrate the spread of various production capacities of the hybridoma population and to show that the assay reagents could identify the secreted antigen-specific antibodies.

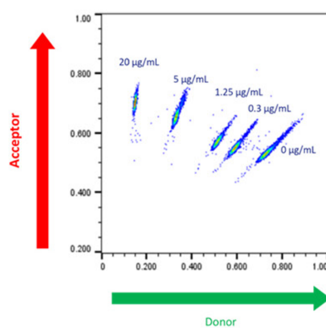


Figure 3. Cyto-Mine® Scatter Plot. A library of picodroplets was made containing different concentrations of target anti-human TNF-alpha IgG and detected using an antigen-specific assay.

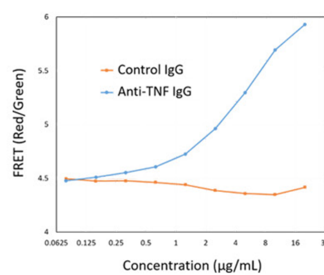


Figure 4. Representative Titration Curve. Generated using the Cyto-Mine® antigen-specific assay. In this example, the control IgG confirms specificity of the assay for human TNF-alpha.

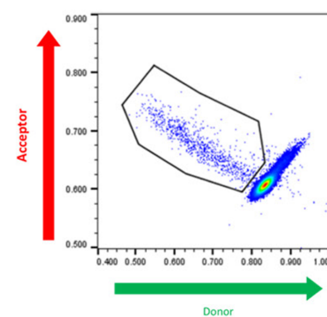


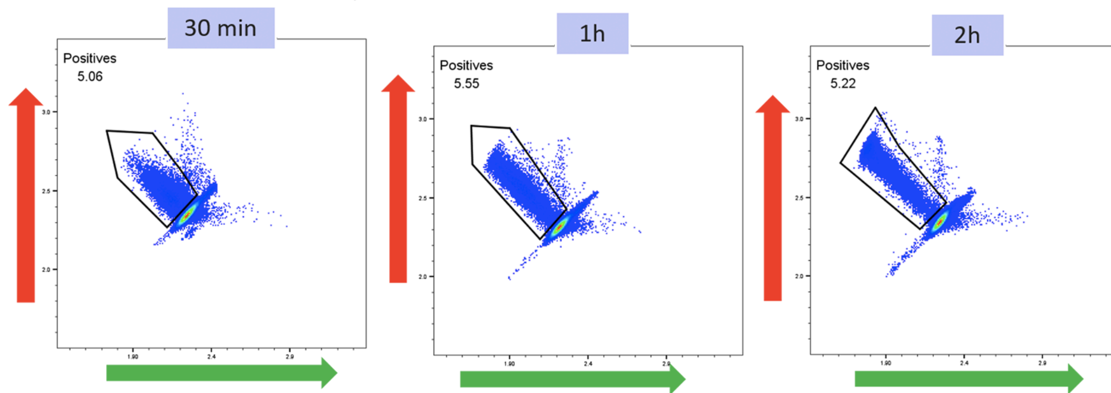
Figure 5. Cyto-Mine® Scatter Plot. FRET signal generated from hybridomas encapsulated in picodroplets and screened for secretion of human TNF-alpha specific IgG.

Cyto-Mine®

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 6 and 7.

Figure 6. Scatter Plot of a Time Course Experiment

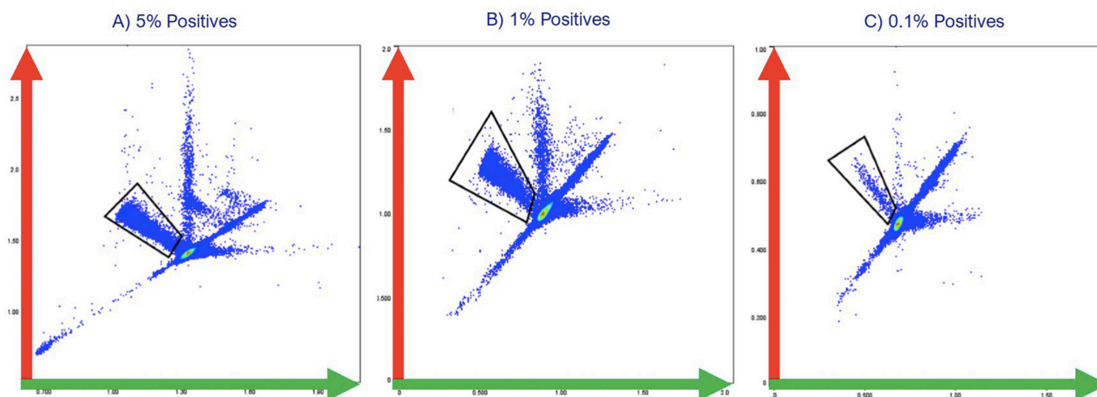


The highest-producing hybridomas appear quickly and early

A longer incubation will enable slower-producing cells to be identified

Picodroplets will become saturated with antibodies and fluorescence with a longer incubation

Figure 7. Detecting Rare Antigen-specific Antibody-secreting Cells in Large Populations



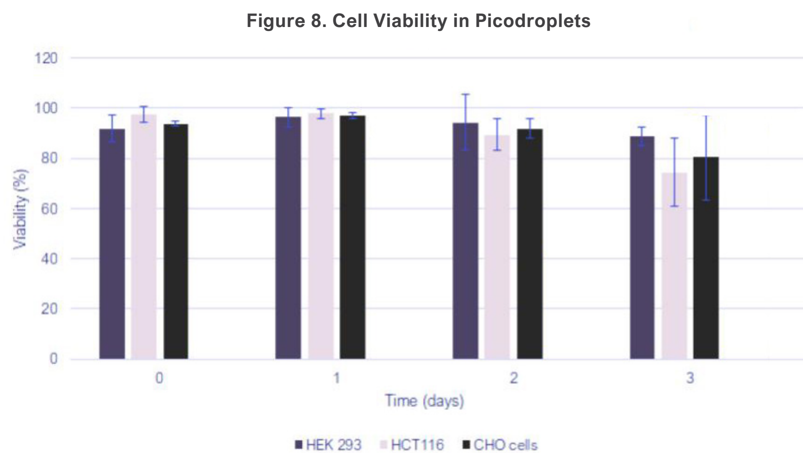
An antigen-specific assay was carried out on 3 mixed hybridoma populations to investigate the sensitivity of Cyto-Mine®. A 'negative' hybridoma population was spiked with varying ratios (0.1% to 5%) of 'positive' hybridoma cells (cells producing the antibody against the target antigen).

CELL-FRIENDLY PROCESSING

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.

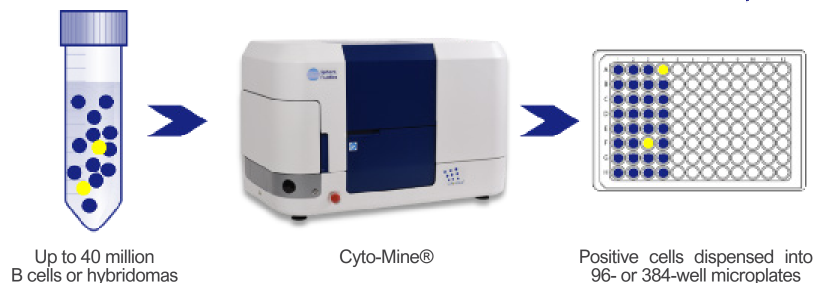
Figure 8 gives a few examples of the types of samples that have been successfully processed through Cyto-Mine® showing that the process is actually quite gentle on the selected cells.

Figure 8. Cell Viability in Picodroplets



SIGNIFICANT TIME SAVINGS

Cyto-Mine® greatly reduces the time it takes to identify hit cells by being able to process up to 200,000 single cells in 1 run or up to 40 million pooled cells in 1 day. By incorporating assay, sorting, imaging and dispensing, Cyto-Mine® allows enrichment of an antibody-producing population, isolation of an antigen-specific antibody-secreting population and supports cell viability.



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