

High-Throughput Antibody Discovery Workflow to Select the Rarest High-Affinity Clones

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INTRODUCTION

The rapid identification of rare high-affinity antibodies among large cell repertoires ($> 10^6$ cells) remains a critical challenge in developing antibody-based therapeutics. The coupling of the D-Sire™ assay (antibody detection on droplet surfaces) with the ModaFlow™ instrument (a new all-in-one microfluidic device) will revolutionize antibody discovery. It offers single-cell high-throughput screening and sorting with precise analysis. This synergy enables more efficient identification of high-affinity antibodies, overcoming the limitations of traditional methods (hybridomas/phage display). Thanks to this innovative, quantitative, and reproducible workflow, the development of new therapies for cancer, autoimmune, and infectious diseases is making a major leap forward, accelerating access to more effective treatments.

THE D-Sire™ ASSAY

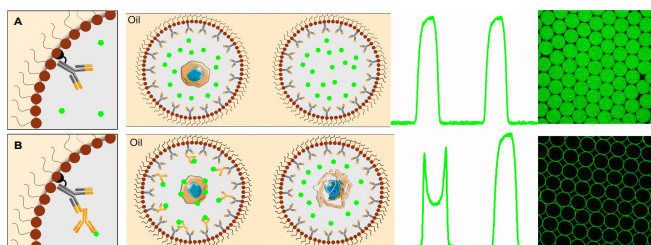


Figure 1: The D-Sire™ Assay. Visualization of the D-Sire system with droplet surface functionalization. A grafting antibody (unlabelled) is present on the surface of all droplets containing the antigen of interest labeled with a fluorochrome (shown in green). **A)** In the absence of secreted antibodies or in empty droplets, the fluorescent antigen remains free in the droplet and there is no fluorescence relocation to the surface. The fluorescence signal appears as a single peak and confocal microscopy visualization shows homogeneous fluorescence within droplets. **B)** In the presence of a cell secreting antibodies such as hybridoma cells, the secreted antibodies are captured at the surface through the grafting antibody coating the droplets. When secreted antibodies have high affinity for the antigen, it gets trapped at the surface of the droplet and the fluorescence signal of positive droplets appears as a U-shape and confocal microscopy visualization shows a fluorescent ring.

- High-throughput single-cell precision: Analyzes secreted proteins at the single-cell level among large repertoires of up to millions of cells.
- Fast response: Results within 2-3 hours.
- Robust microfluidic technology: Versatile and reliable for various applications.
- Rare cell sorting: Identifies and isolates high-value secretory subpopulations.
- Affinity-based sorting: Sorts based on fluorescence signal relocation for more precise selection.

THE ModaFlow™ INSTRUMENT

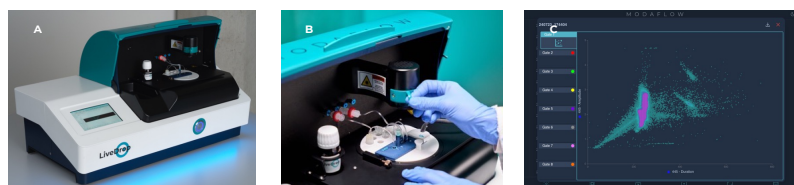


Figure 2: The ModaFlow™ Instrument. **A)** An all integrated and user-friendly microfluidics instrument. Thanks to its lid and a small footprint, the ModaFlow™ fits in a hood and is ideal for sterile work. **B)** Integrates an inverted microscope and a motorised x-y-z stage for precise positioning. Flows are driven by four independent pressure inlets. **C)** Thresholding and gating on up to 5 independent fluorescence channels (up to 4 lasers and 5 photodetectors).

- High-throughput (co-)encapsulation of up to 3 elements.
- Droplet volume range from 15 μm $\varnothing$ (~2 pL) to over 65 μm $\varnothing$ (~150 pL).
- No dead Volume – no sample loss.
- Up to 5 colours multiplexed fluorescence detection.
- Fluorescence activated high-speed sorting ($> 1,000$ events/sec).
- Synchronised imaging of detected/sorted events.
- Up to 50 M droplets/hour (14k droplet/second).

COMPLETE WORKFLOW FOR HIGH-THROUGHPUT ANTIBODY DISCOVERY

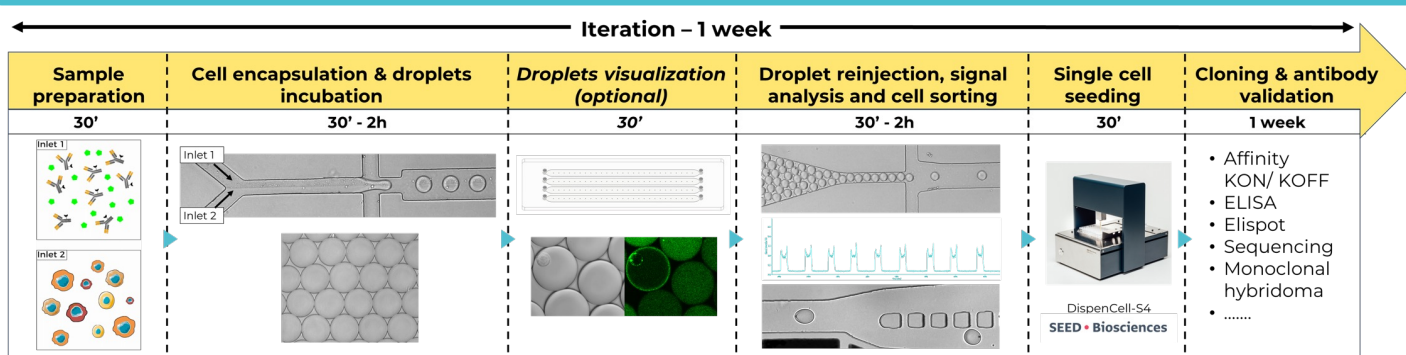


Figure 3: The Complete Workflow for the Discovery of Monoclonal Antibodies. The integration of D-Sire™, an innovative antibody detection assay, with the ModaFlow™, a novel all-integrated microfluidic instrument, offers the possibility to screen antibody secreting cells at very high-throughput, to sort and to isolate high affinity hybridoma clones. A typical workflow starts with the preparation of **samples and reagents** which are **co-encapsulated** in homogenous droplets. Droplets are collected and **incubated for up to 2 hours** while secreted molecules accumulate inside droplets, rapidly reaching detectable concentrations. A small aliquot of droplets can be injected in a **Droplet Visualization Device (DVD)** and observed with a confocal microscope for a visual control. After incubation, droplets are **reinjected** in a sorting chip, the **fluorescence signal is analysed**, and **droplets are selected and sorted** into a collection tube. Cells secreting specific antibodies can be studied in bulk or seeded individually in multi-well plates with the Dispencell™. Finally, clones can be established, and antibodies can be characterized by various techniques including affinity assay (koff/kon), ELISA, ELISpot, sequencing and the establishment of monoclonal hybridoma.

RESULTS & CONCLUSION

Using the workflow described above, we have successfully screened **> 100,000 hybridoma cells**, and more than 120 positive droplets were selected and isolated. After cloning and post-process validation, **more than 100 hybridoma clones** were validated as positive by ELISA, **with a sub nanomolar binding affinity ($> 10^{-9}$ M)**. Droplet microfluidics has emerged as a transformative solution, enabling ultra-high-throughput screening of antibody secreting cells at rates exceeding 100,000 droplets per minute. By encapsulating single cells in picoliter droplets, ModaFlow™ achieves 100% sample utilization. The D-Sire technology proves to be a powerful and versatile tool for screening rare high-affinity antibody-secreting cells. Its application has been demonstrated on various cell types, including PBMCs, B cells, hybridomas and yeasts. This approach enables the specific detection of validated antigens, covering a broad range of proteins (30-400 kDa), as well as other biomolecules such as oligonucleotides, peptides, and lipid-stabilized GPCRs. In conclusion, this user-friendly platform represents a paradigm shift in next-generation antibody development, ultimately improving patient outcomes and advancing the potential of personalized medicine.