CYTO-MINE®: AN INTEGRATED PICODROPLET SYSTEM FOR SINGLE CELL ANALYSIS

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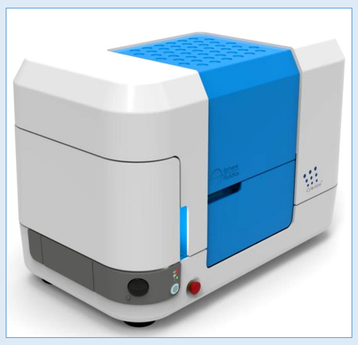
Introduction:

A number of different techniques are routinely used in the biopharmaceutical discovery and development workflow. These include single cell analysis, sorting, imaging and dispensing into individual wells of microtitre plates (MTPs). Traditionally, different instruments would be required for each technique; which is costly, time-consuming and requires extensive lab space that increases the risk of sample contamination. Picodroplet techniques allow for sophisticated and sensitive manipulation of cells at the single cell level. Cyto-Mine® technology is the first integrated device to automatically perform all of these crucial techniques in a single compact system. This high-throughput instrument uses picodroplet technology and microfluidics to process around 1 million heterogeneous mammalian cells in a few hours. Each cell is encapsulated in a picodroplet containing cell culture growth media, which acts as a bioreactor to compartmentalise the cell and let it grow; eventually trapping secreted molecules such as antibodies (Abs). The system also allows label-free selection and deposition of single cells into well of 96- and 384-well MTPs - ensuring high fidelity monoclonality assurance (required for FDA-approval of all monoclonal Abs).

Cyto-Mine® - Key Features:



Cyto-Mine®

- ❑Allows single cell secretion assays.
- ❑Flexibility in assay type and format design.
- ❑Cell friendly sorting.

Screening millions of single cells in < 1 day

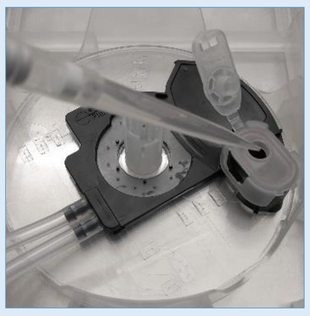
Monoclonality assurance

Specially designed for biopharma

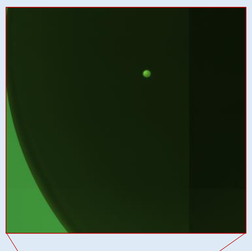
- ❑Rationalized cloning based on measured productivity.
- ❑Cell friendly dispensing into 96- & 384-well microplates.
- ❑Built-in single cell imager prior to dispensing.
- ❑Retrievable and traceable monoclonality data.

- ❑Sterile process and materials.
- ❑Animal component free consumables.
- ❑User friendly.
- ❑Automation compatible.

Cyto-Mine® Workflow:

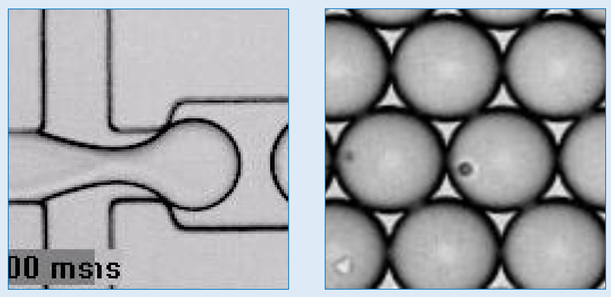


1. User load cells and assay reagents are pipetted into the Cyto-Cartridge®.



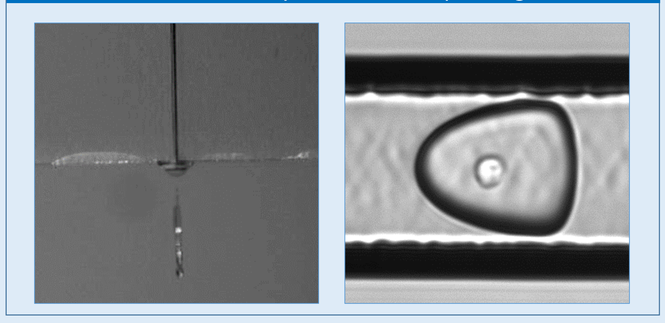
6. Individual cells dispensed in the wells can be directly processed for antibody cDNA retrieval or further cultured to grow into colonies.

Picodroplet Generation & Cell Encapsulation



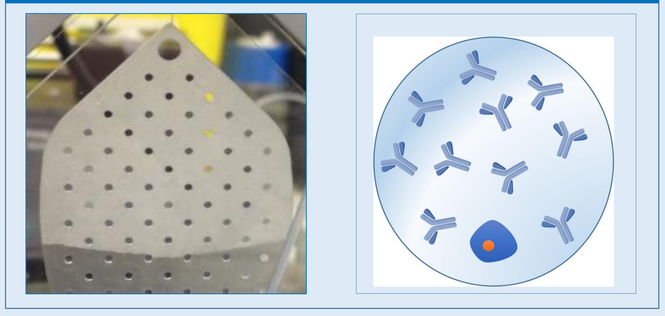
2. Cells and assay reagents are encapsulated in pico-litre-volume picodroplets, together with assay reagents. The distribution of cells in picodroplets follows a Poisson distribution. Hundreds of thousand to several million cells can be encapsulated and processed in a single run.

Monoclonality Assurance Dispensing



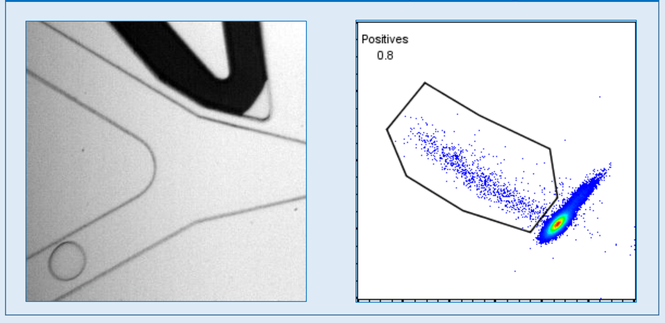
5. Sorted positive picodroplets containing single cells are dispensed one by one into individual wells of 96- or 384 well-plates. Monoclonality is assured by recording multiple images of each picodroplet before they are dispensed.

Incubation & Antibody Secretion



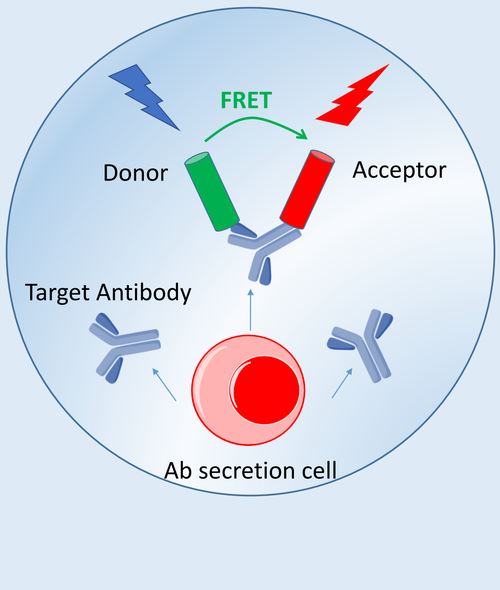
3. Picodroplets containing cells are incubated at 37°C for up to several hours. The cells secrete antibody molecules during incubation. Due to the small volume of the picodroplets, the antibodies produced by a single cell can reach a detectable concentration in a relatively short time window.

Fluorescence-Based Assay & Sorting



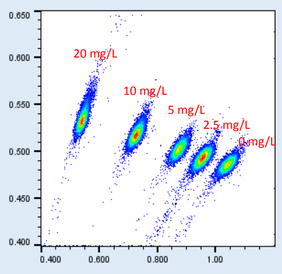
4. The antibodies in picodroplets secreted by single cells can be detected using a FRET-based quantitative fluorescent assay. Positive cells in picodroplets can be sorted based on the gate settings defined by the user.

Example Application Data:

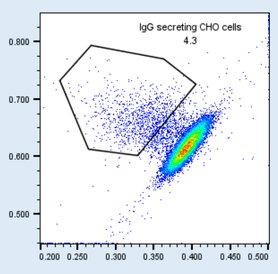


Schematics of FRET-based assays in picodroplets: Förster resonance energy transfer (FRET) based homogenous assays were developed to detect antibodies secreted by cells in picodroplets. The assays use a pair of fluorescently affinity probes that both bind to the target IgG molecules respectively. In presence of the target IgG antibody, the two FRET probes and target IgG molecule bind together, forming a 3-component complex, bringing the FRET donor and Acceptor into close proximity. FRET signal is detected at Acceptor λ(Em) by excitation at donor λ(Ex). A Scatter plot is generated by plotting the fluorescent intensity of acceptor (x-axis) against the donor (y –axis).

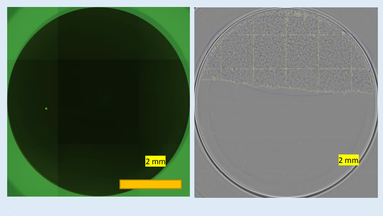
Cell Line Development



Animal component free assay on human IgG standards.

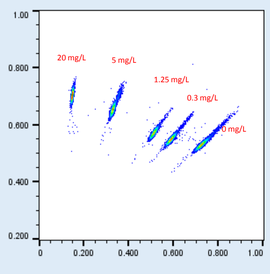


Animal component free assay on human IgG secreting CHO cells.

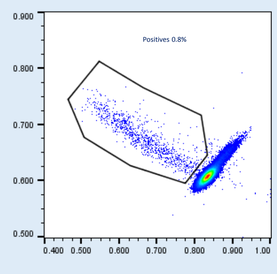


Left: Single cell dispensed into a well in 96-well plate.
Right: A colony grown from a single cell dispensed in the well.

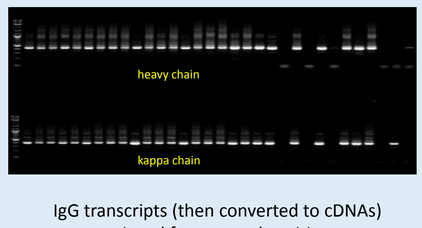
Antibody Discovery



Animal component free assay on mouse IgG standards.



Antigen specific assay on mouse IgG secreting hybridomas.



IgG transcripts (then converted to cDNAs) retrieved from sorted positives.

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