APPLICATION NOTE

Samplix

Revealing and retrieving highly potent IFN-γ secretors using an Xdrop® single-cell format

Mette Juul Jacobsen, Mary Amasia, Christian Hussing, Rikke Stick Højmark, Thomas Mørch Frøsig, Peter Mouritzen, and Marie Just Mikkelsen
Samplix ApS, Birkerød, Denmark

Summary

- Bulk functional assays of immune cells miss highly potent cells within the population.
- This Xdrop single-cell format workflow for IFN-γ secretion assessment reveals the functionality of individual cells, enabling their retrieval and expansion.

Introduction

Bulk functional assays of mammalian cells can only deliver average readouts for the heterogeneous population, masking the view of individual cell activity. This is due to the cross-talk between cells with different activities in the bulk solution.

Samplix has developed Xdrop and the Xdrop DE50 Cartridge to encapsulate living mammalian cells in highly stable double-emulsion droplets (DE50 droplets) for incubation, flow cytometry, and sorting. Here, we show the application for cytokine secretion analysis to reveal highly potent cells that were missed in the bulk assay.

IFN-γ secretion assay

Natural killer cells were labelled with IFN-γ catch reagent according to the Miltenyi Biotec® IFN-γ Secretion Assay protocol. The cells were then resuspended in MEM α with 100 ng/ml IL-2 and FITC-IFN-γ antibody. Half of this activated population was incubated in bulk. We encapsulated the cells of the other half for incubation in double-emulsion droplets using an Xdrop DE50 Cartridge on an Xdrop.

In parallel, non-activated NK cells were prepared: they received the same treatment without exposure to IL-2.

Cells from all four incubations (bulk activated, bulk non-activated, single-cell activated, single-cell non-activated) were analyzed by flow cytometry using a SONY® SH800S cell sorter. Figure 1 shows the Xdrop-based workflow for activated cells.

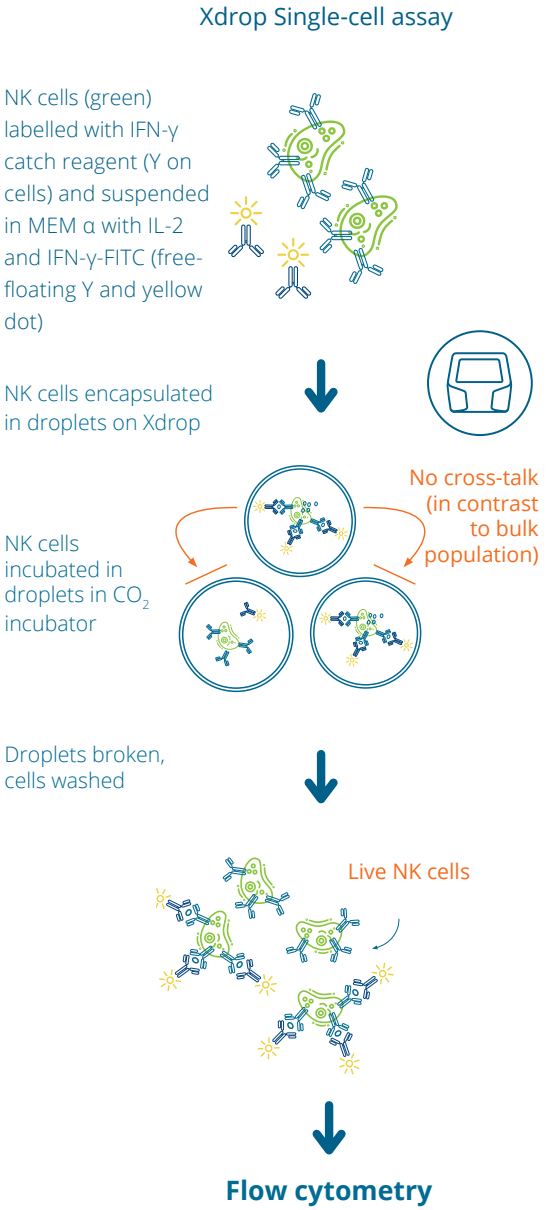

Figure 1. The Xdrop-based workflow for a single-cell format IFN-γ secretion assay

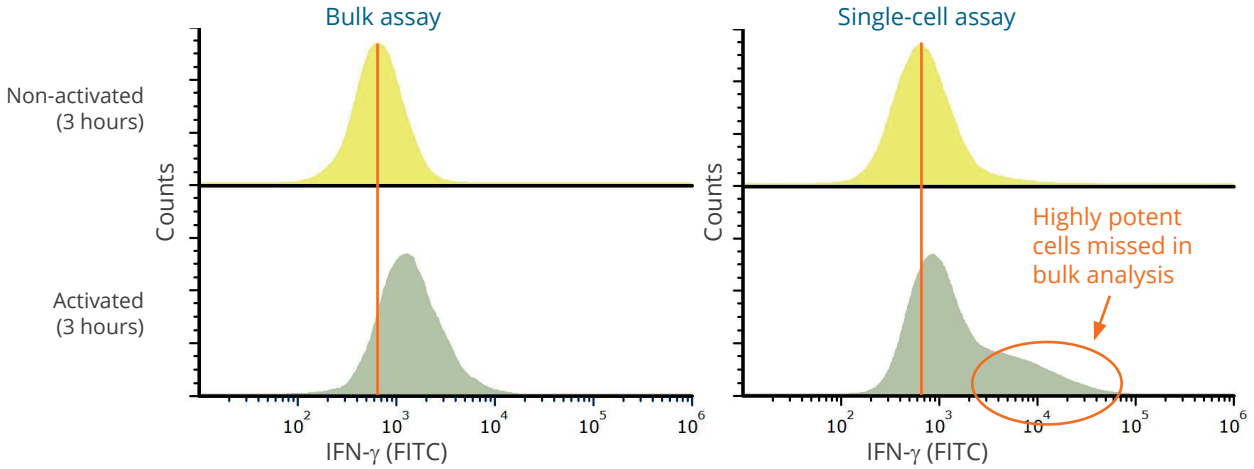

Figure 2. The results for IFN-γ secretion from non-activated and IL-2-activated NK cells incubated for 3 hours in MEM α in bulk (bulk assay) or within double-emulsion droplets generated with an Xdrop DE50 Cartridge on an Xdrop (single-cell assay). The Xdrop-based single-cell format workflow reveals highly potent individual NK cells that are hidden in the averaged readout for the bulk assay.

Retrieval and expansion of cells with IFN-γ activity

NK cells were labelled and activated with IL-2 as described for the IFN-γ secretion assay. Using an Xdrop DE50 Cartridge on an Xdrop, the cells were encapsulated along with FITC-IFN-γ antibody and prodidium iodide (PI) in MEM α. The IFN-γ secretion was assessed using a SONY SH800S cell sorter. Cells with a high IFN-γ secretion profile were enriched via sorting on the SONY instrument and cultured for 2 weeks. In parallel, non–activated cells were cultured for 2 weeks in the same medium.

A second droplet-based analysis of the NK cells' IFN-γ secretion profiles after exposure to IL-2 indicates that those selected for high activation in the first round maintain this high potential over time (Figure 3). The sorted, activated population (Figure 3A) had a higher level of IFN-γ secretion than the non-selected, activated population (Figure 3C) even after incubation.

Conclusion

The Xdrop workflows described here enable the identification of highly potent IFN-γ-secreting NK cells and the successful selection and expansion of those cells. This is a unique approach for cytokine secretion assays, delivering single-cell results for living cells within a short time frame.

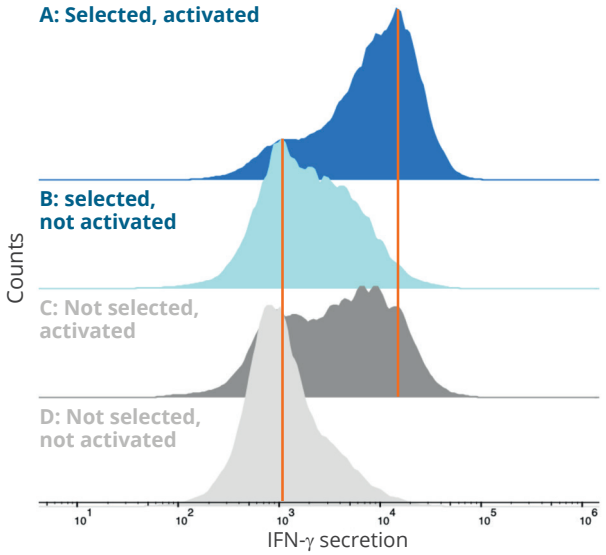

Figure 3. The results for IFN-γ secretion from NK cell populations that were: A and B – previously selected for high IFN-γ secretion, incubated for two weeks, then activated with IL-2 (A) or not activated (B); C and D – incubated for two weeks without any prior activity-based selection, then activated with IL-2 (C) or not activated (D).

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