Revealing and retrieving highly potent IFN-γ secretors using an Xdrop® double-emulsion droplet-based workflow

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Summary

- Bulk functional assays of immune cells miss highly potent cells within the population.
- This Xdrop workflow for IFN-γ secretion assessment reveals these highly potent cells and enables 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.

In cell therapy research, it is critical to have a single-cell view of immune cell activities, e.g., cytokine secretion. Transcriptomics can give this insight, but the cells are killed in the workflow, meaning no possibility for cell recovery or expansion.

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 (NK-92) were washed and 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-92 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 using a BD Accuri™ flow cytometer. Figure 1 shows the Xdrop-based workflow for activated cells. Figure 2 shows the results.

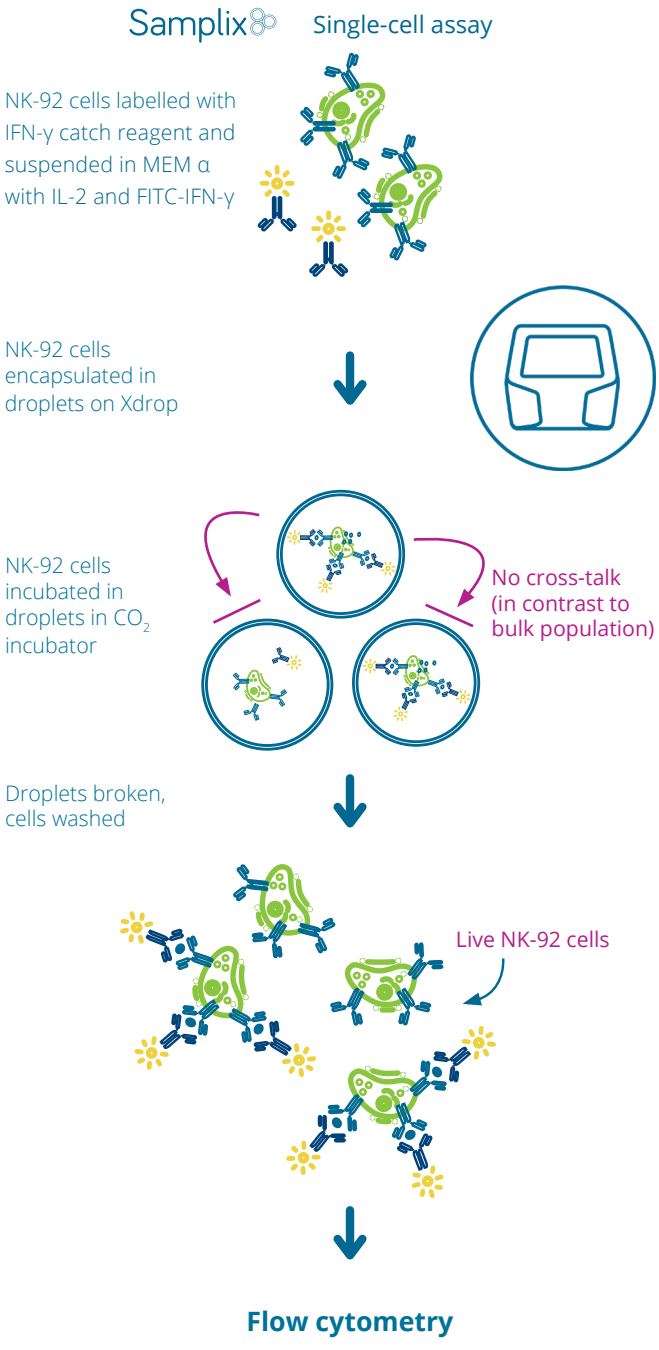

Figure 1. The Xdrop-based workflow for a single-cell IFN-γ secretion assay

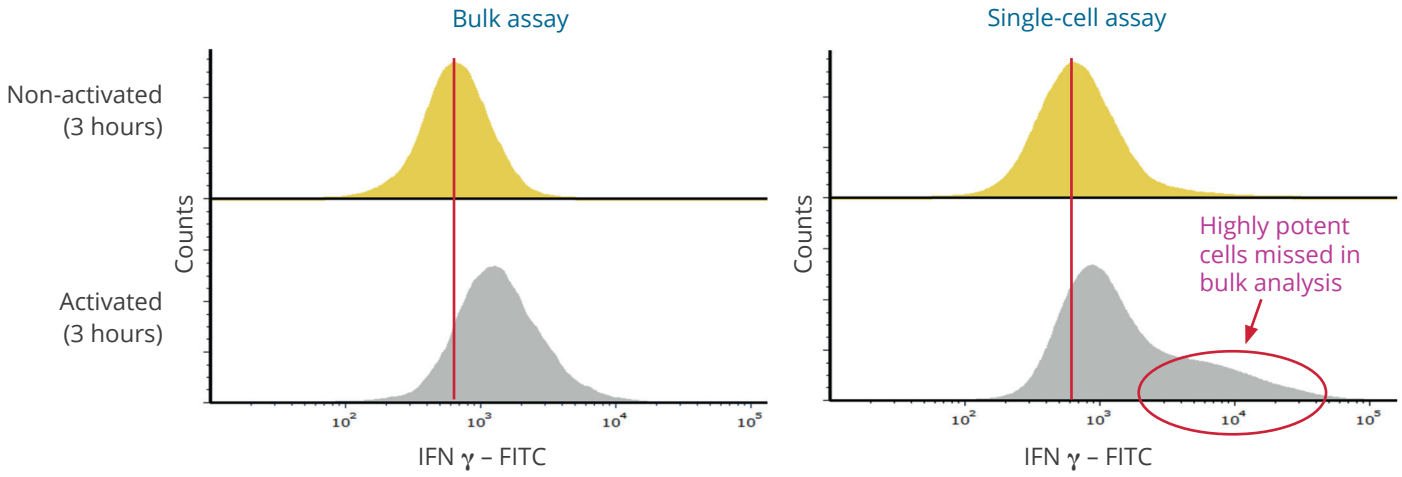

Figure 2. The results for IFN-γ secretion from non-activated and IL-2-activated NK-92 cells incubated for 3 hours in MEM α in bulk (bulk assay) or within double-emulsion droplets generated with an Xdrop DE50 Cartridge on an Xdrop (single-cell assay). The Xdrop-based workflow reveals highly potent NK-92 cells that are hidden in the averaged readout for the bulk assay.

Retrieval and expansion of cells with desired IFN-γ activity

NK-92 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 BD Accuri flow cytometer. Cells with a high IFN-γ secretion profile were enriched via sorting on a SONY® SH800S cell sorter 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-92 cells' IFN-γ secretion profiles after exposure to IL-2 shows that those selected for high activation in the first round maintain this high potential over time (Figure 3).

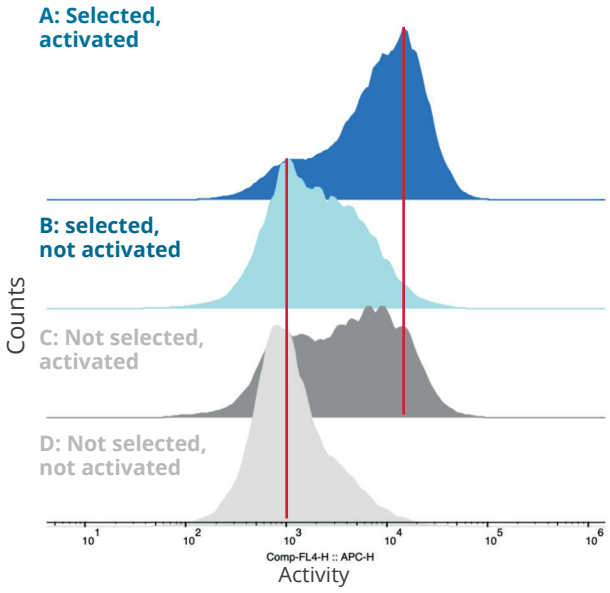

Figure 3. The results for IFN-γ secretion from NK-92 cell populations that were: **A and B –** previously selected for high IFN-γ secretion, incubated for two weeks, then activated with IL-2 (A) or not activated (B); **C and D –** incubated for two weeks without any prior activity-based selection, then activated with IL-2 (C) or not activated (D).

Conclusion

The Xdrop workflows described here enable the identification of highly potent IFN-γ-secreting NK-92 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.

How Xdrop supports functional assays of mammalian cells with single-cell resolution

Using the Xdrop DE50 Cartridge, Xdrop encapsulates living mammalian cells in highly stable, ~100-picoliter, double-emulsion droplets. This can accelerate assays thanks to the picoliter reaction spaces, which force faster cell–cell interactions or cell secretion buildup. Xdrop processes up to 8 samples in parallel, with ~150,000 single-cell assays generated per sample in just 8 minutes. It is possible to incubate cells within droplets in a CO_2 incubator, analyze single cells or droplets on a flow cytometer, and sort and recover cells for expansion and molecular profiling.

Learn more about Xdrop at samplix.com or contact us at samplix.com/contact.

Notes

Xdrop and the Xdrop DE50 Cartridge are for research use only, not for use in any diagnostic procedures.