Rapidly identifying active natural killer cells using an Xdrop® single-cell format assay based on double-emulsion droplets

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Summary

- Bulk assays mask the heterogeneity of individual natural killer cells' actual cytotoxicity.
- This Xdrop single-cell format assay reveals the active natural killer cells in a population and enables their retrieval and expansion.

Introduction

Bulk assays of natural killer cell activity mask the heterogeneity of the individual cells' actual cytotoxicity. Based on the averaged readouts, it is not possible to strictly determine whether, for example, a small or large percentage of the immune cells in the population show killing activity towards the target cells.

In cell therapy research, it is critical to have a single-cell view of immune cell activities, e.g., cell killing. Transcriptomics can provide some insight into this, 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 single-cell format incubation, flow cytometry, and sorting. Here, we show the application for a cell killing assay with rapid identification of the active natural killer cells.

Cell killing assay

After 24 h of stimulation with interleukin 15 (IL15) or 24 h incubation without stimulation, human natural killer (NK) cells were stained with CFSE. Human lymphoblasts (K562 cells) were stained with eFluor670. The cells were then resuspended separately in complete MEM α.

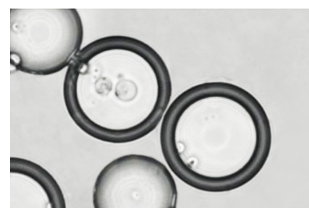
We used the standard Xdrop protocol for co-encapsulation of cells in double-emulsion droplets with an Xdrop DE50 Cartridge and Xdrop Well Insert. The medium was complete MEM α supplemented with propidium iodide (PI) for the detection of dead cells. The ratio for the sample mix was one NK cell to three K562 cells.

The cells were incubated within the droplets for 0, 1, 2, 4, or 24 h at 37°C in 5% CO_2. Figure 1 shows overlaid bright-field and fluorescence microscopy images of a droplet containing both cell types (taken after 24 h of incubation).

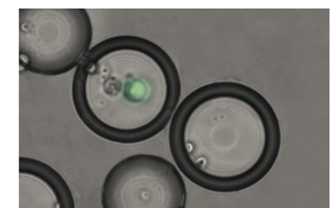
Quantification of droplet content

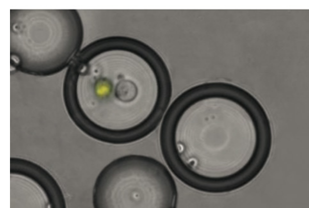
Double-emulsion droplets with co-encapsulated NK and K562 cells were analyzed using a NovoCyte® Quanteon flow cytometer (Agilent® Technologies). The eFluor670 signal (APC-H), representing the droplets containing K562 cells, was plotted against the CFSE signal (FITC-H), representing the droplets containing NK cells. This enabled the gating of droplets containing no cells; K562 cells alone; NK cells alone; and K562 cells together with NK cells (Figure 2, left). The count of droplets with PI-negative (living cells) or PI-positive (dead cells) signals were counted for each gate of the plot (Figure 2, right). The droplets with co-encapsulated K562 and NK cells had a clear shift towards PI-positive (dead) cells compared to the droplets containing only one of the cell types.

Bright field image

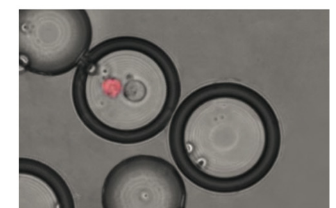


CFSE staining of NK cell





PI staining of dead cell



eFluor670 staining of K562 cell

Figure 1. Overlaid bright-field and fluorescence microscopy images showing the state of cells co-encapsulated in a DE50 droplet. The NK cell is stained with CFSE (green). The K562 lymphoblast is stained with eFluor670 (red). The K562 cell is dead, so it is positive for PI (yellow).

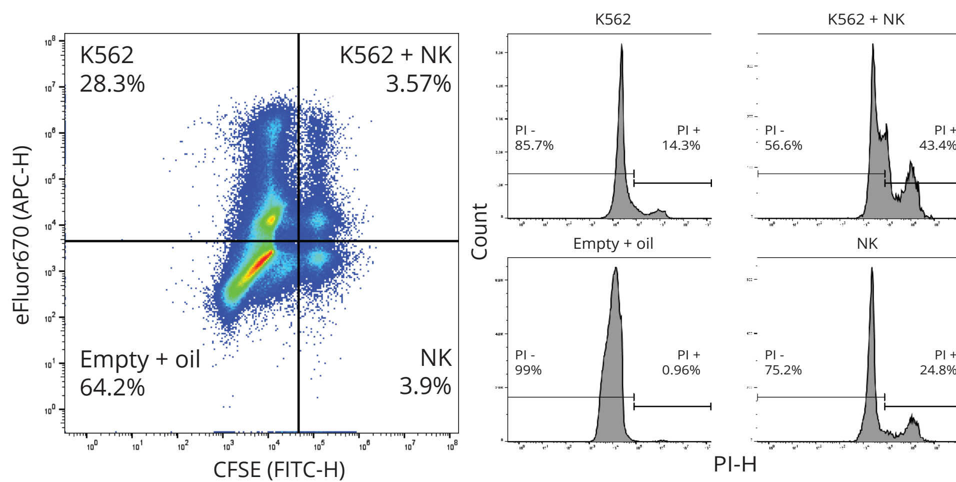


Figure 2. Left: A density plot showing the eFluor670 signal (droplets containing K562 cells) against the CFSE signal (droplets containing NK cells). Right: Histograms showing the PI signal for each of the four gates of the density plot. A PI+ signal indicates the presence of dead cells. All data were compensated with single-stained samples.

Confirmation of cell killing activity

Cell death was determined based on the PI signal from the droplets, which was measured after 0, 1, 2, 4, or 24 hours. Cell killing was determined in the droplets containing both NK and K562 cells after 1, 2, 4, and 24 h by subtracting the background death at 0 h and normalizing to the number of live cells at 0 h. In the IL15-stimulated cultures, cell killing progressed from 3.9% after 1 h to 38.1% after 24 h. In the unstimulated cultures, cell killing was delayed and lower (Figure 3).

In droplets containing only NK cells, there was no increase in cell death during the experiment. In droplets containing only K562 cells, the percentage of dead cells did not increase for the first 4 h. There was a slight increase (7–8%) after 24 h (data not shown).

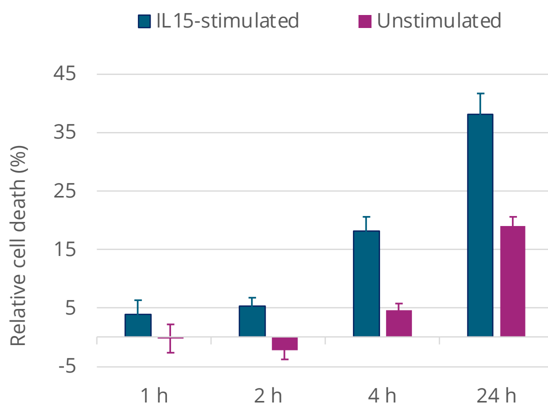


Figure 3. Relative cell death from three experiments +/- SEM. K562 cells and IL15-stimulated or unstimulated NK cells were co-encapsulated in DE50 droplets. The droplets were analyzed using flow cytometry. Relative cell death was calculated by subtracting the background death (PI+ signal for droplets with co-encapsulated NK and K562 cells at 0 h) and normalizing to the number of droplets containing co-encapsulated live cells at 0 h.

Conclusion

The Xdrop workflow for co-encapsulation of immune cells and their targets provides a unique opportunity to rapidly and reproducibly quantify natural killer cells with different activity levels in a population. Target cell killing can be seen within one hour of incubation, i.e., the "fast killers" in the heterogeneous population can be identified. This workflow is an important step forward in the ability to study native and engineered immune cells.

How Xdrop supports functional assays of mammalian cells in a single-cell format

Using the Xdrop DE50 Cartridge, Xdrop encapsulates living mammalian cells in highly stable, ~100-picoliter, double-emulsion droplets. This accelerates assays thanks to the picoliter reaction spaces, which force faster cell–cell interactions and cell secretion buildup. Xdrop processes up to 8 samples in parallel, with ~750,000 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 and 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.

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