

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

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### Summary

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

### Introduction

Bulk assays of killer cell activity mask the heterogeneity of the individual cells' actual cytotoxicity. Based on the averaged readouts, it is not possible to strictly determine if, for example, only a small percentage of the immune cells in the population each killed multiple target cells or if a large percentage of immune cells each killed one or two targets.

In cell therapy research, it is critical to have a single-cell view of immune cell activities, e.g., cell killing. 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 single-cell format incubation, flow cytometry, and sorting. Here, we show the application for a cell killing assay with rapid quantification of the active killer cells.

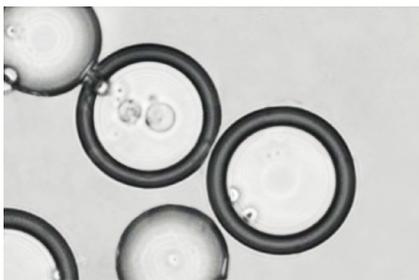
### Cell killing assay

Human lymphoblasts (K562 cells) cultured in MEM  $\alpha$  were stained with eFluor670 and propidium iodide (PI) was added for the detection of dead cells. Human natural killer cells in the same medium were stained with CFSE and PI was also added.

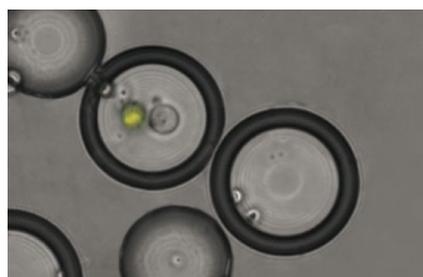
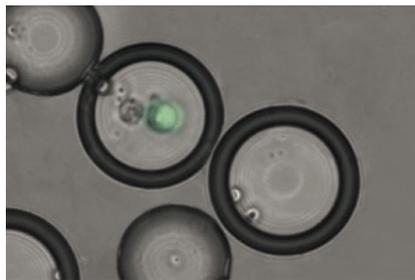
The sample mix had the cells in the ratio of one NK cell to 2.5 K562 cells. We used the standard Xdrop protocol for co-encapsulation of cells in double-emulsion droplets with an Xdrop DE50 Cartridge and Xdrop Well Insert.

The cells were incubated within the droplets for 1, 2, 4, or 24 hours at 37°C in 5% CO<sub>2</sub>, then analyzed using a BD Accuri™ flow cytometer. Overlaid bright-field and fluorescence microscopy images are shown in [Figure 1](#).

#### Bright field image



#### CFSE staining of NK cells



#### PI staining of dead cells



#### eFluor670 staining of K562 cells

**Figure 1.** Overlaid bright-field and fluorescence microscopy images showing the state of cells encapsulated in the double-emulsion droplets. The green stain (CFSE) shows the natural killer cell (NK). The red stain (eFluor670) shows the lymphoblast (K562 cell). The yellow stain (PI) shows that the co-encapsulated K562 cell died, most likely killed by the NK cell. Note that image resolution and brightness have been manipulated for clarity.

## Quantification of droplet content

The count of droplets with eFluor670 signals was plotted against the count of droplets with CFSE signals, enabling the quantification of droplets containing no cells; K562 cells alone; NK cells alone; and K562 cells together with NK cells (Figure 2).

## Confirmation of cell killing activity

Cell death was determined based on the PI signal from the droplets, which was measured after 1, 2, 4, or 24 hours. To confirm that killing activity took place (as opposed to cell death due to other means), the percentage of droplets containing only a dead K562 cell or a dead NK cell was compared to the percentage containing a dead K562 cell with a living NK cell (Figure 3).

As can be seen, significant cell killing activity occurs within the first hour, with around 25% of the co-encapsulated K562 cells dead compared to only 5% of K562 cells that were alone in droplets. After 24 hours, almost 50% of the co-encapsulated K562 cells were dead; and only ~10% of the K562 cells that were alone in droplets.

This method enables the easy identification of active NK cells in a population. Since the DE50 droplets are sortable on fluorescence-based cell sorters, it is also possible to retrieve the active killer cells for subsequent expansion.

## Conclusion

The Xdrop workflow for co-encapsulation of immune cells and their targets provides a unique opportunity to rapidly quantify active killer cells in a population. Results are faster than with a bulk assay thanks to the ~100-picoliter droplet volume, which forces faster cell-cell interactions. Significant target cell killing can be seen within one hour of incubation. This workflow constitutes 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 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<sub>2</sub> incubator, analyze single cells or droplets on a flow cytometer, and sort and recover cells for expansion and molecular profiling.

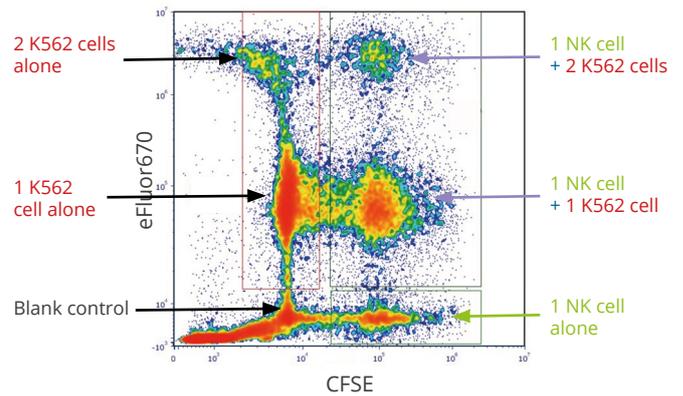
Learn more about Xdrop at [samplix.com](https://www.samplix.com) or contact us at [samplix.com/contact](https://www.samplix.com/contact).

## Notes

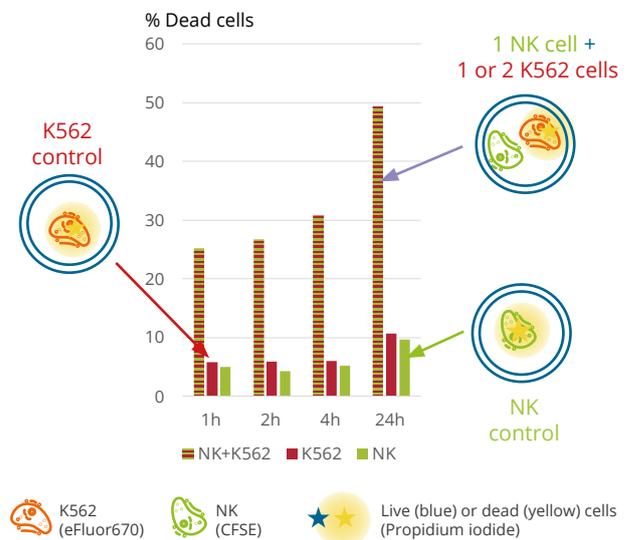
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**Figure 2.** A plot of the count of droplets with eFluor670 signals (i.e., containing K562 cells) against the count of droplets with CFSE signals (i.e., containing NK cells) used to quantify the droplet content. The PI signal was used to determine the level of cell death in the three populations of droplets containing cells.



**Figure 3.** The percentages of dead cells in droplets after 1, 2, 4, or 24 h of incubation based on the PI signal. Cells encapsulated alone were treated as the control for the cell killing assay.