

Identifying highly potent TNF- α -secreting T cells from blood samples in 7 hours using the 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 reveals highly potent TNF- α -secreting T cells in a human blood sample.

Introduction

A single-cell view of immune cell activities, such as cytokine secretion, is critical to cell therapy research. However, most common secretion analyses use a bulk workflow, where cross-talk between secreting and non-secreting cells creates a readout bias (Figure 1, lower workflow). What's more, the overnight incubation that can be required can mean cytokines build up in the medium, giving a falsely high secretion reading.

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. The workflows provide the required single-cell view of activity levels.

Here, we use the Xdrop workflow for TNF- α secretion analysis (Figure 1, upper workflow), identifying and

isolating highly potent T cells that would be missed in a bulk assay. What's more, the workflow does not require an overnight incubation, allowing completion within a working day and avoiding falsely high readings.

TNF- α secretion assay

Human PBMCs (peripheral blood mononuclear cells) from a healthy donor were used for a TNF- α secretion assay based on the Xdrop workflow and a bulk assay.

The PBMCs were labeled with TNF- α capture reagents according to the Miltenyi Biotec[®] TNF- α Secretion Assay protocol. Then, the population was split into four groups. The cells of one group were encapsulated in DE50 droplets together with TNF- α PE antibody and 1 μ l/ml cytokine stimulation cocktail. The cells of the second were also encapsulated with TNF- α PE antibody but without 1 μ l/ml cytokine stimulation cocktail. The third and fourth groups were non-encapsulated cells respectively with or without 1 μ l/ml cytokine stimulation cocktail.

All cells were then incubated (in droplets or in bulk) at 37°C in 5% CO₂ for 1, 2, and 4 hours. TNF- α PE antibody was added to the bulk groups after incubation.

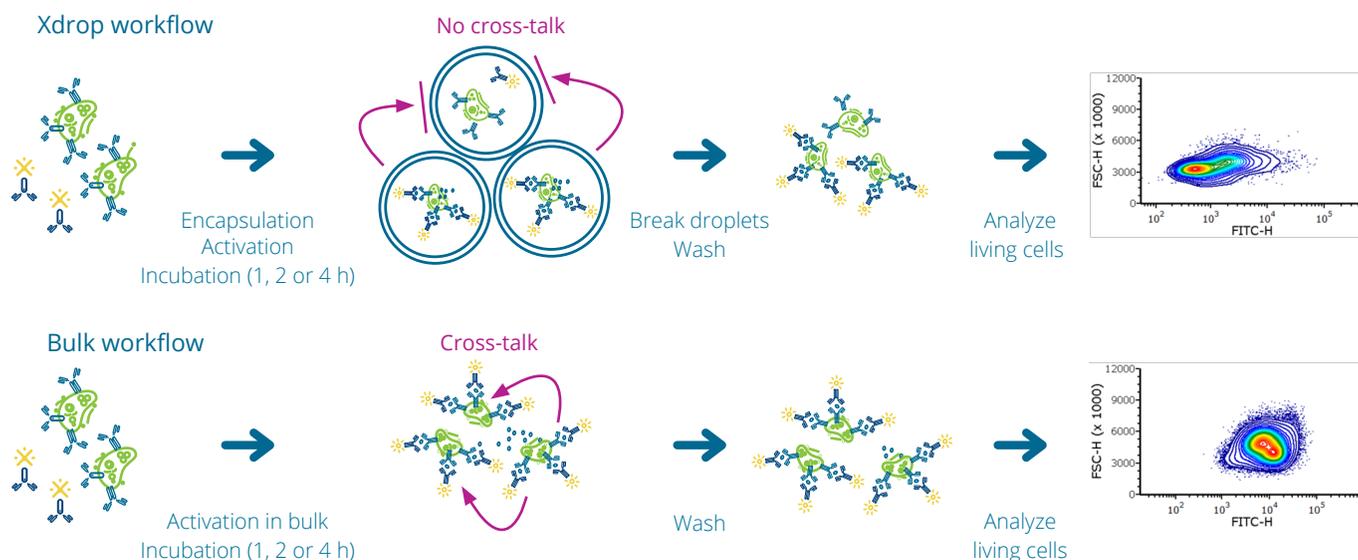


Figure 1. The Xdrop workflow for a single-cell TNF- α secretion assay with 1, 2, or 4 hours of incubation (top) and the equivalent bulk assay with the same incubation times (bottom). The whole Xdrop workflow takes 5 to 7 hours depending on the incubation time. Set up: 1 h 20 min. Droplet generation: 8 min. Incubation: 1–4 h. Droplet breaking and staining: 1 h. Analysis on flow cytometer: 5 min/per sample. While this version of the bulk workflow takes the same amount of time, it does not deliver equivalent results.

The cells from the first two groups were then released from the droplets and all cells were washed in phosphate-buffered saline (PBS) with 2% fetal bovine serum (FBS), stained with live/dead-FITC stain and CD3⁺-PerCP, and analyzed using a BD Accuri™ flow cytometer. The T cells in the samples were specifically detected via gating based on the CD3⁺-PerCP signal.

Figure 1 shows the Xdrop-based and bulk workflows for the activated cells. Figure 2 shows the TNF- α secretion profiles of the T cells from the blood samples.

Highly active TNF- α -secreting T cells are detected after 2 hours of incubation in droplets

After 1 hour of incubation, the TNF- α secretion profiles for the activated T cells from the bulk and Xdrop groups looked similar to each other (Figure 2A).

After 2 hours of incubation, the profile for the activated T cells from the two workflows were distinctly different, with a subpopulation of highly potent cells clearly present in the Xdrop group (Figure 2B). After 4 hours, this difference was even more pronounced (Figure 2C). At no point is such a subpopulation of highly potent T cells discernable from the bulk activated profile.

What's more, the peak value for the bulk activated group increases significantly over the four hours, while the peak value for the Xdrop activated group only increases by a relatively small amount.

These results are indicative of cross-talk between the cells in the bulk activated group.

Conclusion

The Xdrop workflow using the Xdrop DE50 Cartridge allows for precise, single-cell TNF- α secretion analysis for human T cells in a population of PBMCs within an 8-hour working day, including an incubation time of just 2 to 4 hours. Overnight incubation is not necessary. The results clearly show how significant a population of highly potent secretors can be hidden in the results of a bulk assay.

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 CO₂ 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

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

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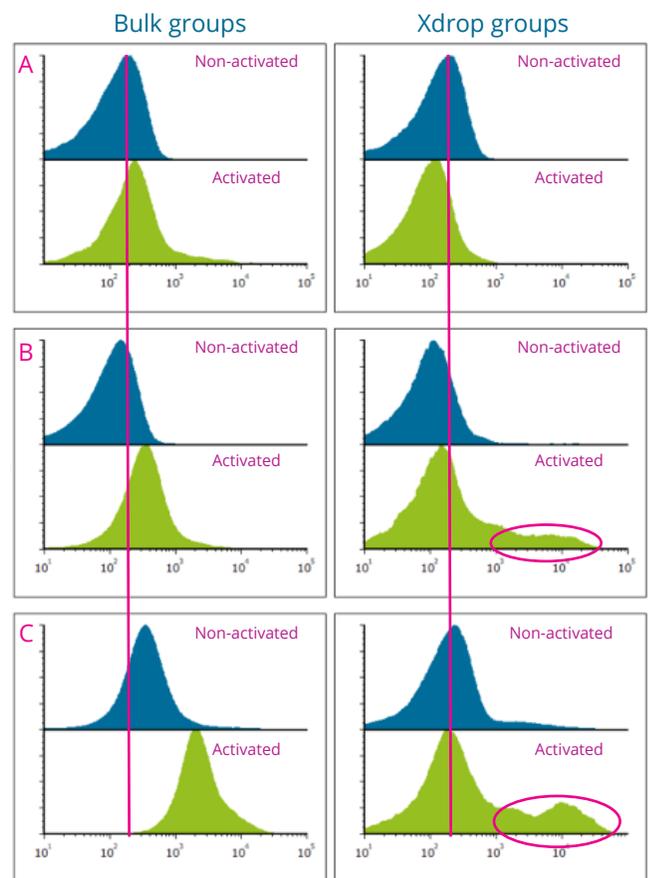


Figure 2. The TNF- α secretion profiles of the T cells from the blood samples. The cells were processed in four groups: activated and non-activated bulk groups; and activated and non-activated Xdrop groups (cells encapsulated in DE50 droplets). Y-axis: cell count. X-axis: TNF- α . **A** The results after 1 hour of incubation. **B** The results after 2 hours of incubation. Note the circled area of the profile, indicating highly potent T cells. **C** The results after 4 hours of incubation. Note the circled area is larger and the peak for the bulk activated group is significantly different.