APPLICATION NOTE

Samplix

Quantifying cells that secrete cytokines using the Xdrop® single-cell format

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Summary

- This Xdrop workflow enables rapid, multiplex quantification of the individual immune cells in a population that are secreting one or more cytokines.
- The results for single-cell format multiplex and singleplex assay concurred and were reproducible.

Introduction

Bulk cell analyses can miss crucial information on the heterogeneity of immune cell populations and the diversity of individual immune cell responses. Within cell therapy research, there is a clear need for rapid, sensitive, and reliable functional analyses of cells with single-cell resolution to gain these critical insights.[1]

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.

The Xdrop workflow provides the required single-cell view of cytokine levels, because the droplet encapsulation prevents crosstalk between the cells. It allows for the analysis of 150,000 single cells per sample with the option to recover immune cells with the desired functionality for later molecular profiling or further cell expansion.

Furthermore, results are obtained in less than eight hours. This is thanks to the small droplet volumes (~100 pl), which ensure secreted cytokines rapidly reach detectable levels.

Here, we use the Xdrop workflow to encapsulate immune cells in droplets for the quantification of TNF-α and IFN-γ secretion and digital counting of the number of actively secreting cells. We also show that the workflow results are highly reproducible.

Experimental setup

Human natural killer cells (NK cell line) and a population of human peripheral blood mononuclear cells (PBMCs) from a healthy donor were analyzed in parallel.

The NK cells and PBMCs were respectively labeled with IFN-γ and TNF-α capture reagents according to the Miltenyi Biotec® IFN-γ and TNF-α Secretion Assay protocols. The cell populations were divided in two: a non-activated control subpopulation that was not exposed to cytokine stimulants; and an activated population that was exposed to 25 ng/ml cell stimulation cocktail (PMA/Ionomycin), 100 ng/ml IL-2, and 2.5 µg/ml PHA-M. This was earlier shown to activate the NK cells and the T cells among the PMBCs (data not shown).

The control and activated subpopulations of each cell type were then divided into samples of ~460,000 cells (6,700 cells per µl medium) for encapsulation in DE50 droplets together with IFN-γ-APC and TNF-α-PE antibodies.

The cells within the DE droplets were subsequently incubated for 3 hours at 37°C in 5% CO_2. After incubation, all the cells were recovered from the droplets, washed, and stained with LIVE/DEAD Fixable Green Dead Stain (Thermo Fisher Scientific®) to identify living cells. The PBMCs were also stained with CD3-PerCP to identify the T cells within the

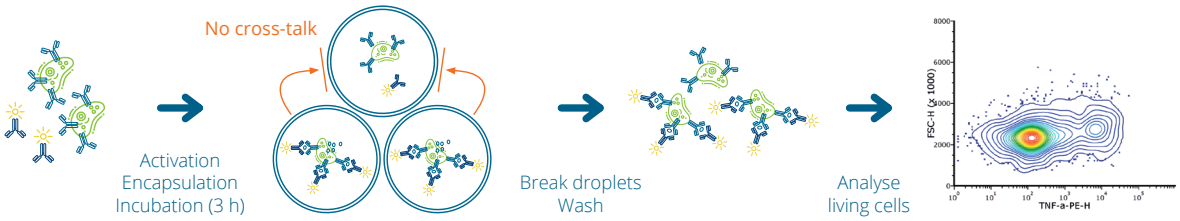

Figure 1. The Xdrop workflow for a single-cell cytokine secretion assay. The whole workflow takes ~6 hours based on the 3 hours of incubation used here. Two to four hours of incubation has been found to be sufficient in other experiments (data not shown).

population. All the cells were then analyzed using a BD Accuri™ flow cytometer.

To verify that the multiplex assay yields comparable results to a singleplex assay, the experiment was repeated for each cell type and each cytokine singly, i.e., with one capture reagent bound to the cells instead of two.

The reproducibility of this Xdrop workflow for cytokine secretion analysis was determined by repeating the same experiments 5 times on 3 different days (Figure 2).

Figure 1 shows the workflow for a single-cell secretion assay.

Highly active TNF-α and IFN-γ secretors are reproducibly detected after 3 hours of in droplets

We retrieved 5 replicates of the TNF-α and IFN-γ secretion profiles from non-activated (control) and activated NK cells and Tcells. Figure 2 shows the box plot for the 5 replicates, discriminating between cells secreting TNF-α, IFN-γ, or both of these cytokines.

As expected, non-activated cells display a very low secretion profile, while activated cells show a high profile. The activated T cells mainly secreted TNF-α (45%) and the activated NK cells IFN-γ (82%). Interestingly, almost all the T cells that secreted IFN-γ also secreted TNF-α, and almost all the NK cells that secreted TNF-α also secreted IFN-γ. The standard deviation of <5% illustrates the very high reproducibility.

Figure 3 shows the consistent results for the singleplex and multiplex versions of the droplet-based single-cell assay.

Conclusion

The Xdrop workflow allows for cytokine secretion profiling with precise measurements at the single-cell level. The results are highly consistent and reproducible, and it is possible to investigate one or more cytokine secretion profiles simultaneously and within one working day.

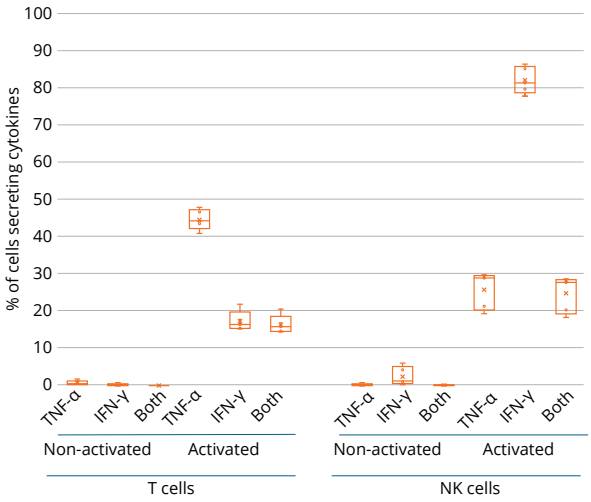

Figure 2. Box plot showing the TNF-α and IFN-γ secretion profiles for non-activated (control) and activated T cells within a PMBC population from a human blood sample and for non-activated (control) and activated NK cells. Data derived from 5 replicates performed on 3 different days.

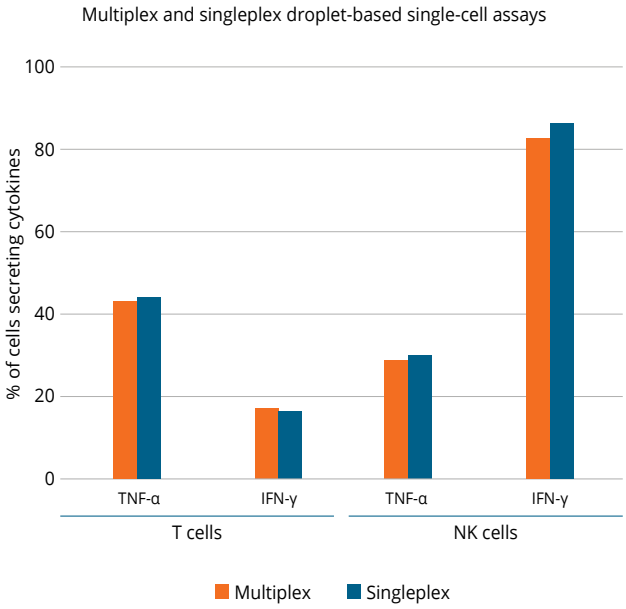

Figure 3. Clustered histogram showing the percentages of NK cells and T cells secreting TNF-α and IFN-γ in multiplex and singleplex versions of the Xdrop workflow.

For more information about Xdrop products and applications, visit samplix.com.

References

1. European Medicines Agency. 2016. Guideline on potency testing of cell-based immunotherapy medicinal products for the treatment of cancer. Technical update downloaded from www.ema.europa.eu in August 2022