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Xdrop® DE20 and DE50 droplets enable consistent results in cell functional analyses and cell viability after incubation

Christian Hussing, Mary Amasia, Rikke Stick Højmark, and Marie Just Mikkelsen
Samplix ApS, Birkerød, Denmark

Summary

- Double-emulsion droplets produced with Xdrop (DE20 and DE50 droplets) are uniform in size and shape.
- The droplets display a desirable specific permeability to fluorophores and compounds.
- The Samplix reagents and gentle droplet production with Xdrop ensure high cell viability in the droplets.

Introduction

Samplix has developed Xdrop and the Xdrop DE20 and DE50 Cartridges for the generation of double-emulsion droplets (DE20 and DE50 droplets, respectively). They can be used to encapsulate:

- Living mammalian and microbial cells for incubation, flow cytometry, sorting, and other analyses
- Nucleic acid fragments, proteins, and other molecules for highly targeted analyses.

DE20 and DE50 droplets offer a unique approach for convenient, high-throughput, accurate functional analyses of individual cells, e.g., protein secretion and killing activity assays. They are compatible with flow cytometers and cell sorters thanks to their stability and aqueous outer phase. Moreover, they can be incubated in standard CO_2 incubators with common culture media.

To draw meaningful conclusions from experiments, it is essential that the droplets are monodisperse and produced in a reproducible manner.

Here, we show the results of analyses of DE20 and DE50 droplets regarding variation in inner droplet diameter, droplet permeability to fluorophores and other compounds relevant in single-cell analysis, and viability of cells within droplets. These results show that Xdrop technology provides the conditions needed for single-cell functional analysis in droplets.

Inner droplet diameter varies by less than 5.5%

DE20 and DE50 droplets were produced using an Xdrop and Xdrop DE20 or DE50 Cartridges as described in Xdrop Manual (version 6.1 or later). Table 1 shows the reagents used in the cartridges.

For each droplet type, we ran six droplet productions with three different cartridges, each from a different batch. After the production, 2 µl of the droplets were transferred to a counting chamber and the sizes of five individual droplets from each production were measured in a bright-field microscope view.

The inner diameter of the droplets was found to vary by less than 5.5% (Figure 1): a variance of 0.8 µm for the DE20 droplets (5.4%) and 1.3 µm for the DE50 droplets (2.2%).

Table 1. Reagents for DE20 and DE50 droplet production for the inner droplet diameter assessment. DE PCR mix, Droplet oil DE, DE PCR buffer, and Xdrop DE Oil I are provided by Samplix.

Type	Inner phase	Oil	Outer phase
DE20	DE PCR mix	Droplet oil DE	DE PCR buffer
DE50	MEM α and 10% OptiPrep™ with 12.5% FBS and 12.5% horse serum	Xdrop DE Oil I	Same as inner phase

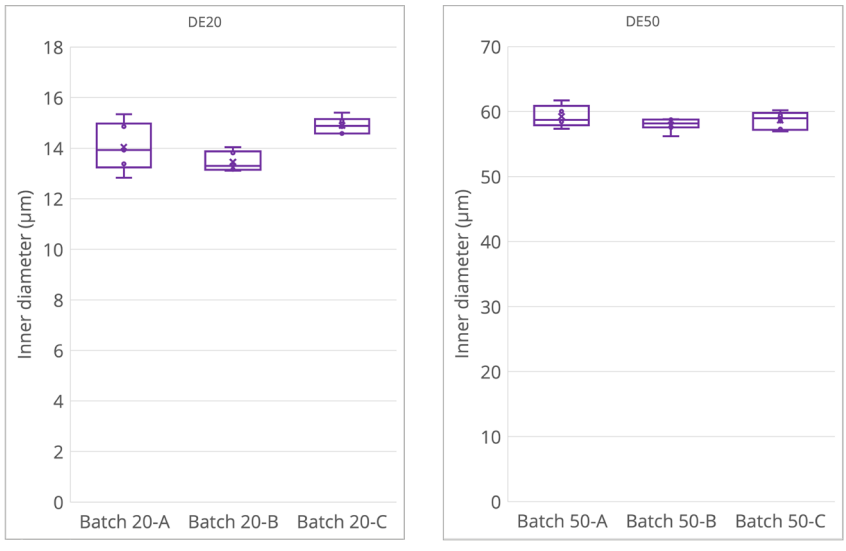

Figure 1. Variation in the inner diameters of DE20 droplets (A) and DE50 droplets (B) produced using 3 randomly chosen Xdrop DE20 or DE50 Cartridges from 3 separate production batches. The variation is less than 5.5% for DE20 droplets and less than 2.3% for DE50 droplets.

Droplet permeability depends on water solubility

Permeability to fluorophores was tested using DE20 droplets produced with four fluorophores (Table 2) added to both the inner and outer phases (DE PCR mix and DE PCR buffer, respectively). After production, the outer phase was exchanged with DE PCR buffer without the fluorophore. Permeability to the fluorophore was measured based on reduction in the fluorescence intensity of the droplets, due to osmotic exchange with the outer phase. Measurements were done with a BD Accuri™ flow cytometer. The halflife was calculated based on the decrease in intensity. Permeability to a selection of other compounds was also assessed (Table 2).

We have not observed any differences between DE20 and DE50 droplets as regards permeability (data not shown).

The permeability of DE20 droplets to the tested fluorophores was found to vary greatly (Table 2), with lower permeability to those that are highly soluble in water.

Table 2. Permeability of DE20 droplets to fluorophores and other compounds

Compound	Permeability	Halflife (if assessed)	Solubility in water at 20°C
Fluorescin sodium salt	Low	>35 days	500 g/l
Resorufin	Medium	~12 min	1 g/l
Syto24	High	<5 min	Very low
Propidium iodide	Low to medium		1 g/l
DNA	Low	>6 months	High
Antibodies	Low		High
Cytokines	Low		High
Dextrans	Low		High
Gases (CO_2, O_2)	High		High

Conclusion

Xdrop DE20 and DE50 products are consistent in size. They allow gases, which are necessary for cell viability, to cross the oil shell but not selected fluorophores and large hydrophilic compounds, such as DNA and proteins. This enables efficient and reproducible analysis of compounds secreted from single cells within the droplets. Droplets with cells of interest can be recovered using cell sorters (data not shown). Thanks to the gentle encapsulation method and specifically designed reagents, high cell viability is seen even after hours of incubation.

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Mammalian cells show high viability in DE50 droplets

Cell viability was tested by encapsulating Ramos B cells (100,000 per sample well in the Xdrop DE50 Cartridge) in DE50 droplets. Table 3 shows the production reagents.

Table 3. Reagents for the encapsulation of Ramos B cells in DE50 droplets for a cell viability study.

Inner phase	Oil	Outer phase
RPMI 1640 20% FBS 2 mM L-Glutamine 100 U/ml Pen/strep 10% OptiPrep Propidium iodide Cells stained with CFSE	Xdrop DE Oil I or Oil B (from a different company)	RPMI 1640 20% FBS 2 mM L-Glutamine 100 U/ml Pen/strep 10% OptiPrep 1x Stabilizing solution for cells

The cells were incubated within the DE50 droplets in a CO_2 incubator and analyzed in a BD Accuri™ flow cytometer after 3 and 22 hours (Figure 2). Viability was determined based on the CFSE fluoresence signal (living cells) and the propidium iodide fluoresence signal (dead cells).

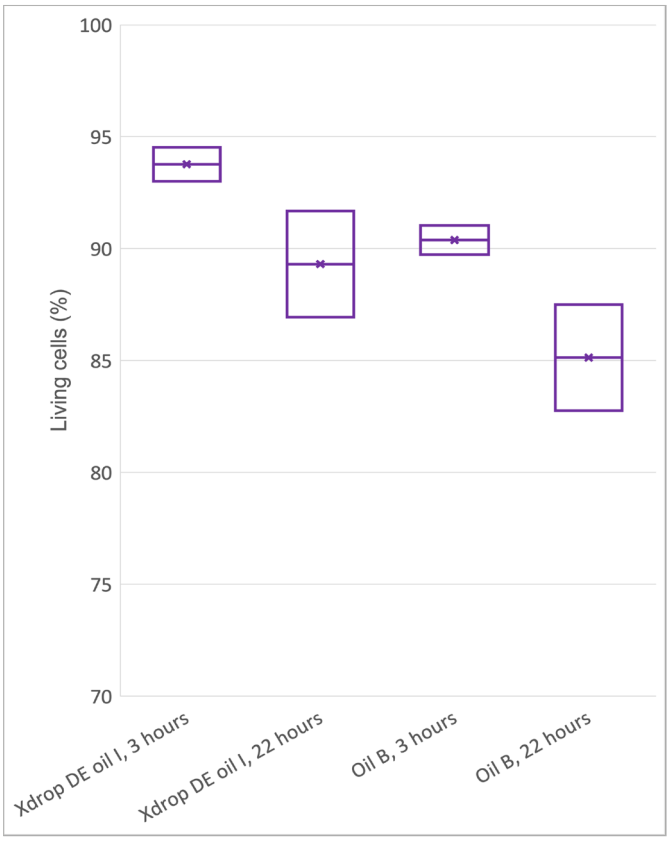

Figure 2. Viability of Ramos B-cells incubated for 3 and 22 hours in DE50 droplets generated with Xdrop DE Oil I or a fluorocarbon oil from another supplier. All datapoints were replicated three times. As can be seen, after 22 hours in DE50 droplets formed with Xdrop DE Oil I, the cells maintained a high viability of almost 90%. When the oil from the other supplier was used, the viability decreased to around 85% after 22 hours.