EVALUATION AND COMPARISON OF THE PERFORMANCE OF FLUOSURF™-S AND FLUOSURF™-O FOR DROPLET STABILITY ALONG THERMOCYCLING

FluoSurf™-S and FluoSurf™-O are high-performance fluorinated surfactants designed and optimized to stabilize aqueous droplets in fluorinated oils for a wide range of biotechnological applications. They are especially adapted for droplet-based microfluidic experiments such as droplet digital polymerase chain reaction (ddPCR).
This document presents the results of an experimental study comparing the performance of FluoSurf™-S and FluoSurf™-O for droplet stability along thermocycling.

• EXPERIMENTAL CONDITIONS

For each surfactant, droplet generation was performed 3 times under the following conditions:

- **Oil phase** : 4w/w% FluoSurf™-S or FluoSurf™-O in HFE-7500
- **Aqueous phase** : 100 µM rhodamine 6G in PBS
- **Chip** : PDMS/glass chip treated with Fluo-ST2

After collection, droplets were submitted to thermocycling (30 or 40 cycles):

Step	Amplification program	Number of cycles
1	30s at 98°C	1
2 3 4	10s at 98°C 5s at 50°C 10s at 72°C	30 or 40
5	∞ at 10°C	1

Table 1 : Standard amplification program

After thermocycling, droplets were injected in a microfluidic chamber for observation. Statistical analysis of droplet size was performed by image analysis (ImageJ). The coefficient of variation (CV) is calculated as the ratio between the standard deviation and the mean droplet size.

• RESULTS

The coefficients of variation calculated after 30 and 40 PCR heating cycles and direct reinjection are shown in the figure below.

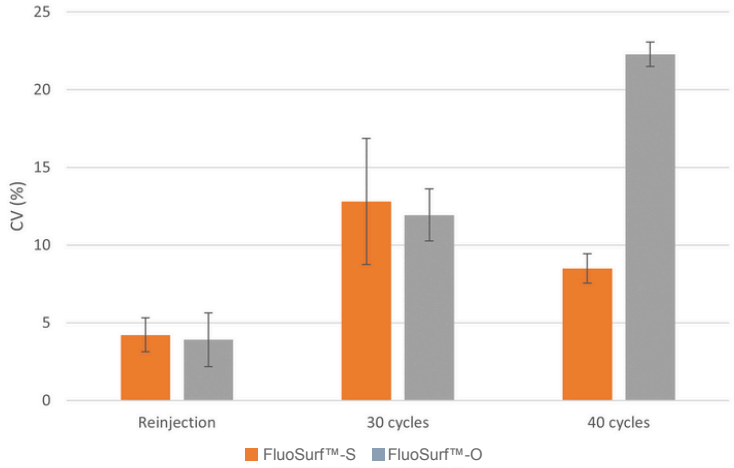

Figure 1 : Coefficients of variation determined through image analysis

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Monodisperse populations are obtained after direct reinjection (CV < 5%) for both populations of droplets generated with FluoSurf™-S and FluoSurf™-O.

After 30 PCR heating cycles:
The coefficient of variation of droplets generated with FluoSurf™-S is 12.8 ± 4.0 %, while the one of droplets generated with FluoSurf™-O is 11.9 ± 1.7 %.
→ **FluoSurf™-S and FluoSurf™-O show similar performances in stabilizing droplets after 30 PCR heating cycles.**

After 40 PCR heating cycles:
The coefficient of variation of droplets generated with FluoSurf™-S is 8.5 ± 1.0 %, whereas the one of droplets generated with FluoSurf™-O is 22.3 ± 0.8 %.
→ **We are showing here that FluoSurf™-S allows a better stabilization of droplets after 40 PCR heating cycles than FluoSurf™-O.**

The same experiments were also carried out with Fluo-Oil 135, our alternative oil to HFE 7500, as fluorinated oil. The coefficients of variation of droplets generated with FluoSurf™-S after 30 and 40 PCR heating cycles are 17.3 ± 0.9 % and 13.1 ± 2.5 % respectively.
→ **FluoSurf™-S is also effective at stabilizing droplets generated in Fluo-Oil 135 during 30 and 40 PCR heating cycles.**

FluoSurf™-S and FluoSurf™-O both allow a good droplet stabilization after 30 PCR heating cycles, whereas FluoSurf™-S is more effective to stabilize droplets after 40 PCR heating cycles in HFE-7500 and Fluo-Oil 135 oils.

To learn more about surfactants and other formulation products for droplet-based microfluidics, please visit www.emulseo.com

Parc AMPeRIS, Bâtiment BAYA, 8 rue Adrienne Bolland 33600 Pessac, FRANCE
Email: contact@emulseo.com / Tel: +33 (0)5 35 54 10 06 / Website: www.emulseo.com