Sphere Fluidics

Pico-Break™

An innovative solution for the phase separation of emulsions

www.spherefluidics.com

What is Pico-Break™ 1?

Picodroplet technology is a rapidly growing area of interest and has many potential applications. This technology is particularly important where tests need to be conducted with only a few nanolitres or picolitres of sample containing, for example, cells or biologically-relevant solutions, such as proteins. As a result, picodroplet technology enables scientists to perform thousands to millions of simultaneous reactions.

Pico-Break™ 1 is a specialised chemical solution used to induce phase separation of an aqueous emulsion stabilised with Pico-Surf™ 1. It contains a proprietary, orange-coloured, fluorocarbon dye in 1H, 1H, 2H, 2H-perfluoroctan-1-ol (PFOH) that is soluble in fluorophilic solvents and can be used as a phase contrast reagent.

Table 1. Pico-Break™ 1 is available in two standard (10 mL and 50 mL) pre-made solutions.

Product Name	Volume	Product Type	Product Code
Pico-Break™ 1	10 mL	Emulsion breaking solution	C081
Pico-Break™ 1	50 mL	Emulsion breaking solution	C082

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Key Benefits of Pico-Break™ 1

- **Easy and reproducible:** phase separation of any emulsion stabilised with a Pico-Surf™ 1.
- **Clear visualization:** as the colouration makes it easy to distinguish the two liquid phases.
- **Suitable for many applications**: e.g. recovery of cells from picodroplets.
- **Efficient recovery:** of biomolecules and cells from the aqueous layer.
- **Quality:** rigorous QC and QA testing including NMR to ensure consistency and reproducibility.
- **Confidence:** we provide experienced scientific and application support.

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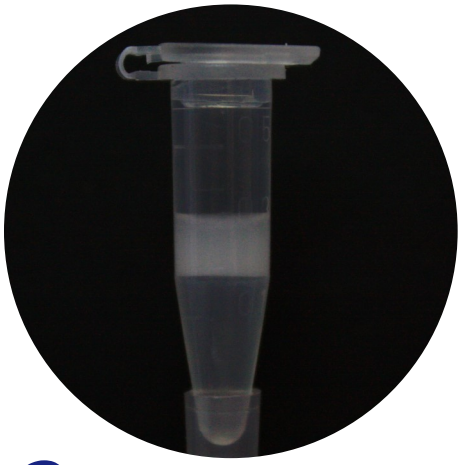
How do I use Pico-Break™ 1?

Before starting, please ensure the emulsion has been collected in, or transferred to, a micro-centrifuge tube.

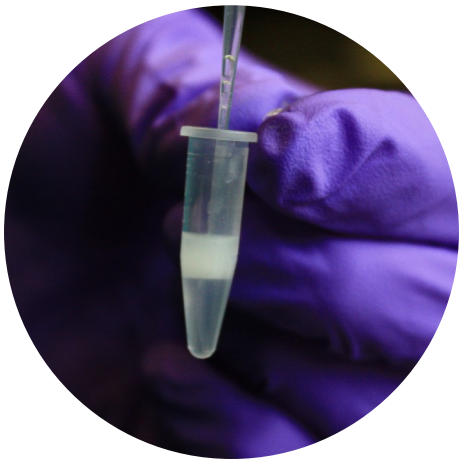


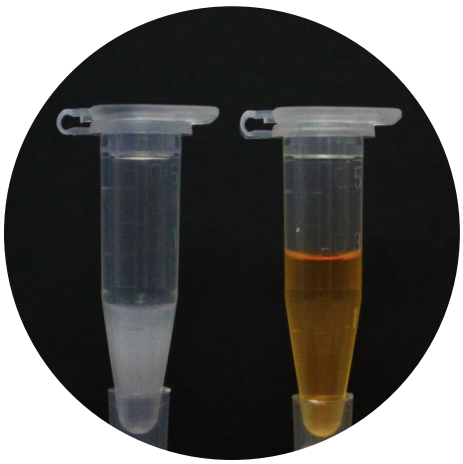
1. After generating picodroplets with Pico-Surf™ 1 you should have a micro-centrifuge tube containing Pico-Surf™ 1, oil and emulsion. Keep the micro-centrifuge tube upright to ensure that the emulsion is floating on top of the Pico-Surf™ 1.

If required, cool the oil and emulsion to approximately 4°C by storing in a refrigerator or ice bath.

2. For easy extraction use a thin, gel-loading pipette tip, alternatively a standard plastic pipette tip will suffice. Carefully remove as much of the Pico-Surf™ 1 oil (bottom layer) as possible. This will minimize the amount of Pico-Break™ 1 that you will need to use to break the emulsion.

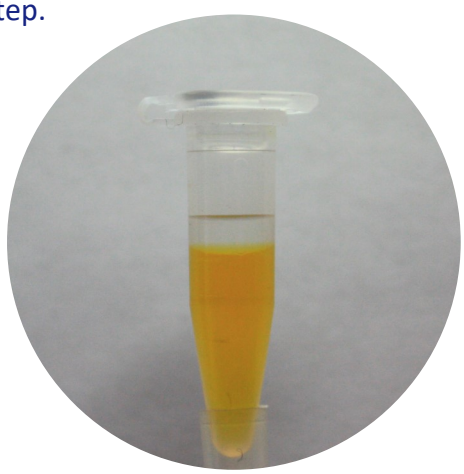
Quick tip: Do not fully depress the pipette before entering the oil. Once the pipette tip is in the bottom oil layer fully depress the pipette to blow any emulsion, that may have been transmitted through the layers, away from the pipette tip.





3. Estimate the volume of Pico-Break™ 1 needed to break the emulsion (see 'How Much Pico-Break™ Should I Add To My Emulsion?').

Add Pico-Break™ 1 to the emulsion, close the lid of the tube and gently agitate the mixture by inverting. You should see the emulsion start to disperse and turn orange. Check that the emulsion has broken before moving to the next step.



4. Centrifuge the mixture in a micro-centrifuge for 30-60 seconds (this is dependent on the sample) at RCF 100-1000 *x g* to completely disperse the emulsion and separate the two phases.

N.B. If the emulsion contains mammalian cells, centrifuge the sample at 100 *x g* for 5 seconds immediately after the addition of Pico-Break™ 1 and agitate.

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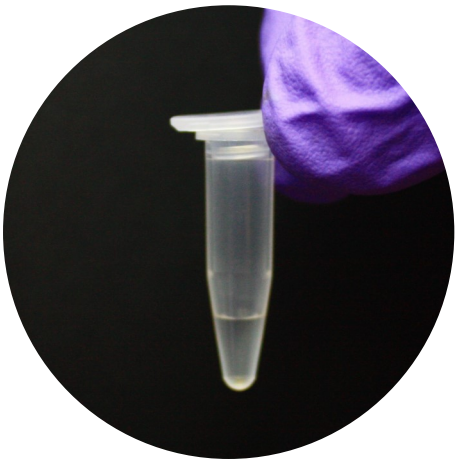
5. After centrifugation, phase separation should have occurred, and two layers should be seen. The bottom orange-coloured layer is the unwanted fluorous phase. The top phase contains your sample of interest.

Tilt the tube 45° and using a pipette carefully remove the top aqueous layer and transfer to a clean micro-centrifuge tube for analysis.



6. The aqueous phase is now ready for further experimentation.

How much Pico-Break™ 1 should I add to my emulsion?

After the removal of Pico-Surf™ 1 oil, a general rule, for the volume of Pico-Break™ 1 required to cause phase separation of the emulsion, is as follows:

- Use **two times the total volume** of emulsion/fluorous oil for Pico-Surf™ 1 concentrations below 3%.
- Use **three times the total volume** of emulsion/fluorous oil for Pico-Surf™ 1 concentrations above 3%.

Table 2. The volume of Pico-Break™ 1 required to induce phase separation of an emulsion based on the percentage of Pico-Surf™ 1 used to generate the emulsion.

Percentage of Surfactant Concentration	0.5 %	1 %	2 %	3 %	4 %	5 %
Volume of Pico-Surf™ 1 (µL)	100	100	100	100	100	100
Volume of Emulsion (µL)	100	100	100	100	100	100
Volume of Pico-Break™ 1 (µL)	**200**	**200**	**200**	**200**	**300**	**300**

Effect of Pico-Break™ 1 on Cell Viability

At Sphere Fluidics, we understand that single cells are fragile and require careful handling after the gentle isolation of picodroplets. Consequently, we examined the effect of Pico-Break™ 1 on cell viability to analyze if there was any compromise in cell integrity.

Method

The effect of Pico-Break™ 1 on cell viability was examined using CHO-S cells. CHO-S cells were stained using a green CMFDA (5-chloromethylfluorescein diacetate) fluorescent dye (live cells) and a far-red fluorescent nuclear stain, DRAQ7™ (dead cells), following manufacturer's instructions.

Half of the population of cells were encapsulated in 300 pL picodroplets and analysed using the Picodroplet Single Cell Encapsulation System, under the conditions outlined in Table 3.

Table 3. Conditions for picodroplet generation.

Parameter	Condition
Aqueous flow rate	1000 µL/h
Fluorinated oil – Pico-Surf™ 1 Novec 7500, 5%	1400 µL/h
Frequency	1000 Hz

Results

To recover the cells from the picodroplets, the picodroplets must be de-emulsified.
Firstly, the majority of fluorous oil was removed from the bottom of the micro-centrifuge tube. The emulsion of picodroplets was then broken by adding Pico-Break™ at three times the total volume of Pico-Surf™ 1 Novec 7500, 5%. The micro-centrifuge tube containing the emulsion was quickly centrifuged and the aqueous phase containing the cells was removed and transferred to a microtitre plate. Both the non-encapsulated control cells and the cells exposed to encapsulation and Pico-Break™ 1 were assayed for viability. The percentage of live and dead cells present in the culture was analyzed using a fluorescence plate reader.

Figure 1. The application of Pico-Break™ 1, to CHO-S cells encapsulated with Pico-Surf™ 1 did not show any significant effect on cell viability after use (cells were treated immediately after picodroplet generation, time 0 hours).

The novel, emulsion breaking solution, Pico-Break™ 1, was shown to retain cell viability of CHO-S cells encapsulated in picodroplets preserving cell integrity for further single cell analysis.

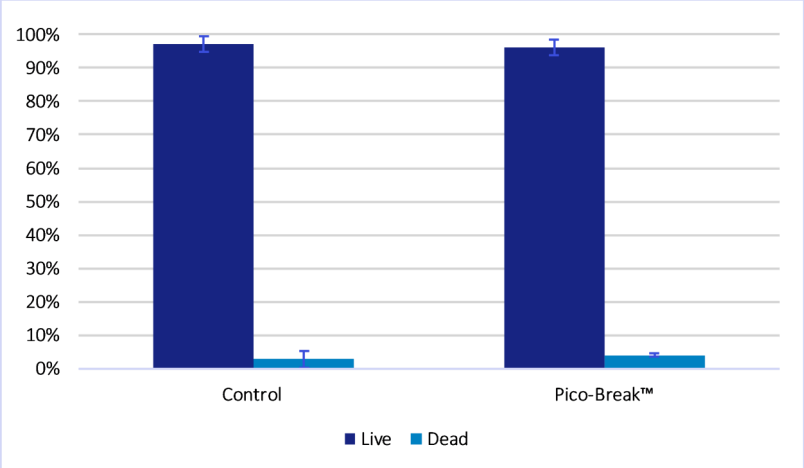


Figure 1. the control cells were 97% viable in comparison to 96% viability recorded from CHO-S cells treated with Pico-Break™ 1 immediately after picodroplet generation.

Instrumentation also available

Cyto-Mine®

The Single Cell Analysis and Monoclonality Assurance System

Selective screening, cell isolation and clone verification integrated into a single platform. Reduce your timelines, increase screening capability and delivery monoclonality. Accelerate your biologics discovery and cell line development workflows.

Research Instruments

- **Picodroplet Single Cell Encapsulation System**
- **Picodroplet Single Cell Assay and Isolation System**

Enabling you to generate, sort, and retrieve picodroplets for a range of applications. Both are compatible with our range of microfluidic specialist chemicals and biochips, as well as other standard and custom biochips from other sources.

Notes:

Sphere Fluidics Ltd is an ISO 9001:2015 accredited company.
Pico-Surf™ and Pico-Break™ 1 are trademarks of Sphere Fluidics Ltd.
Novec™ is a trademark of 3M.
All Sphere Fluidics' supplied chemicals and bioreagents are Animal Origin Free and GLP-compliant.
For research purposes only.
Product specifications subject to change without notice.

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