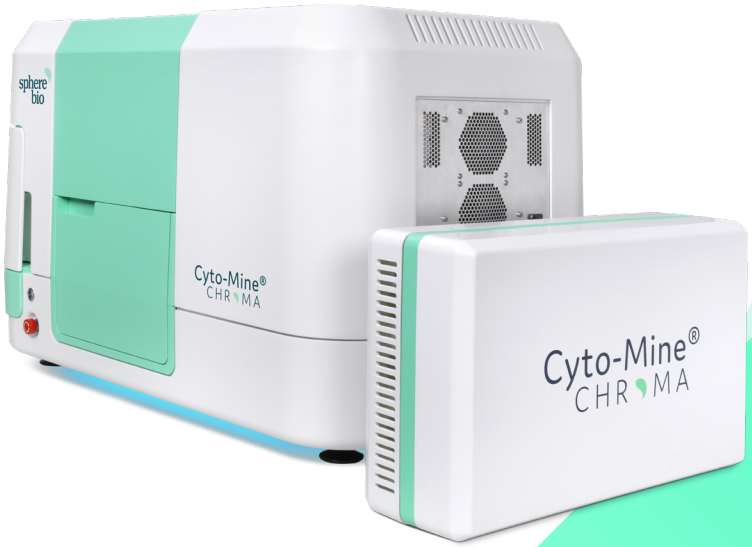
Customer Protocol

Pooled Dispensing Procedure Using Pico-Break™ with Cyto-Mine® Platforms





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1. Introduction

This protocol describes how to perform pooled dispensing and recovery of picodroplet contents from Cyto-Mine® and Cyto-Mine® Chroma platforms. Pooled dispensing involves dispensing multiple droplets into a single container rather than one droplet at a time. It enables the enrichment of target cell populations, including high-secreting, antigen-specific, or secreting B cells, supporting the isolation of cells with desirable functional characteristics for downstream applications. Following pooled dispensing, picodroplets are transferred into a recovery workflow using Pico-Break™. By gently reducing picodroplet stability, Pico-Break™ facilitates the release and recovery of viable cells while maintaining cell integrity for subsequent culture, expansion, enrichment, or further analysis.

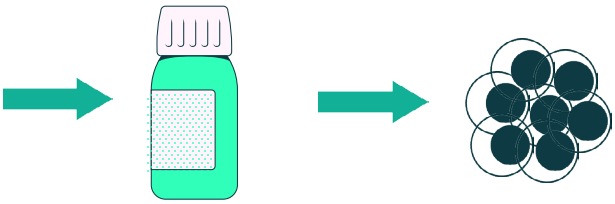
When performed as described, this protocol provides a simple and reliable method for recovering viable cells from pooled picodroplet populations generated on Cyto-Mine® and Cyto-Mine® Chroma systems, enabling efficient enrichment and progression of selected cell populations into downstream workflows.



Cyto-Cartridge®

Cyto-Mine® or Cyto-Mine® Chroma

Cells dispensed into 24-well plate



De-emulsification using Pico-Break™

Pooled Cell Population for Downstream Processing

If you have any questions or need our advice when carrying out this protocol, please contact our support team at support@fluidic.com and we'll be happy to help.

2. Equipment and Materials

Equipment

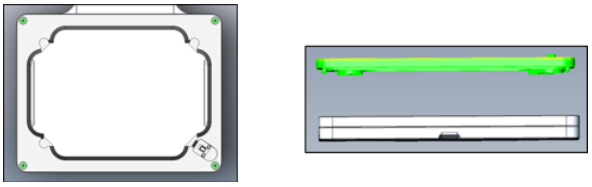
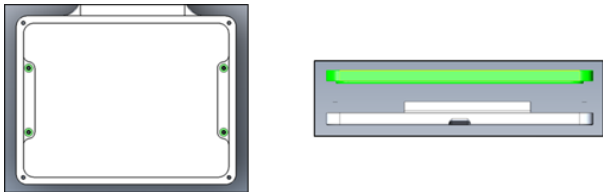
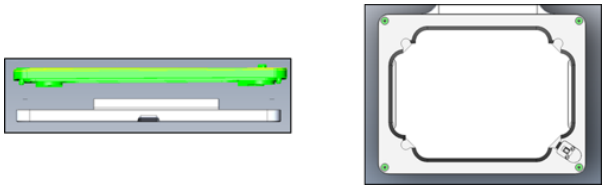
Item	Supplier	Catalogue Number
Cyto-Mine® or Cyto-Mine® Chroma	Fluidic Sciences	S003 or S008
2mm Hex Key	Various	
2.5mm Hex Key	Various	
Centrifuge	Various	

Materials and Reagents

Product Name	Supplier	Catalogue Number
OptiPrep™ Density Gradient agent	Sigma-Aldrich	D1556
Cyto-Surf® A	Fluidic Sciences	C303
Pico-Break™	Fluidic Sciences	C081 or C082
Culture medium (fresh)	Various	
24-well microtiter plate	Various	
5 mL microcentrifuge tube (conical bottom)	Various	
15 mL centrifuge tube	Various	

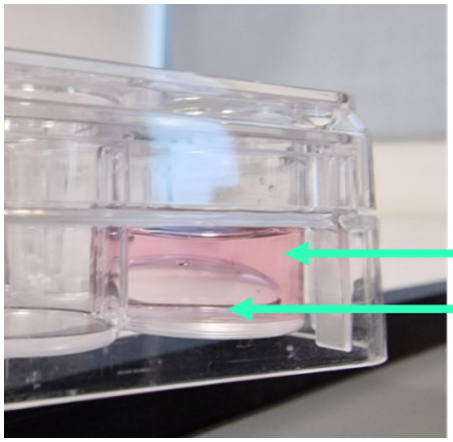
3. Method

3.1 Cyto-Mine®/ Cyto-Mine® Chroma Preparation

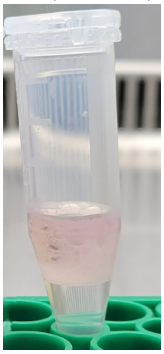
Step	Operation	Details
1	Modify the plate stage to enable configuration for a 24-well microtiter plate	• Before beginning an experiment, from the Main Menu use the Plate Operation button to eject the microtiter plate stage. • Using a 2 mm Hex Key, unscrew the four securing screws and remove the well plate holder.  • Using a 2.5 mm Hex Key, unscrew the four securing screws and remove the clamp plate spacer. Keep it in a safe place.  • Refit the well plate holder using the four-plate holder securing screws 

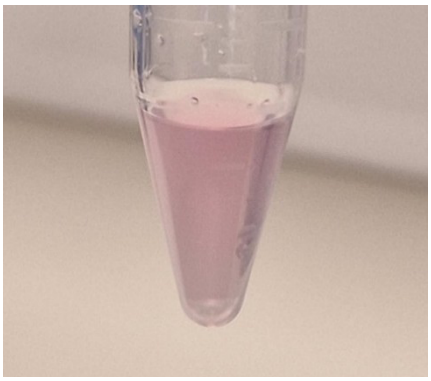
3.2 Pooled Droplet Dispensing

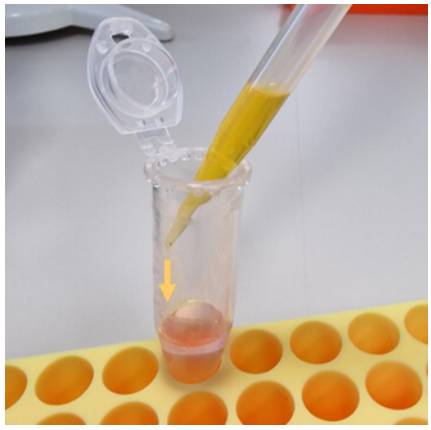
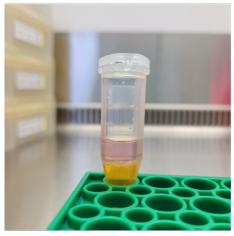
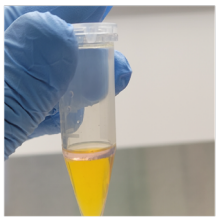
Step	Operation	Details
1	Prepare the 24-well microtiter plate	• Fill the first well (A1) with 1 mL fresh culture medium containing 16% (v/v) OptiPrep™ and without serum. ***Note:*** *Opti-Prep™ prevents the cells from sedimenting. Serum may affect de-emulsification*

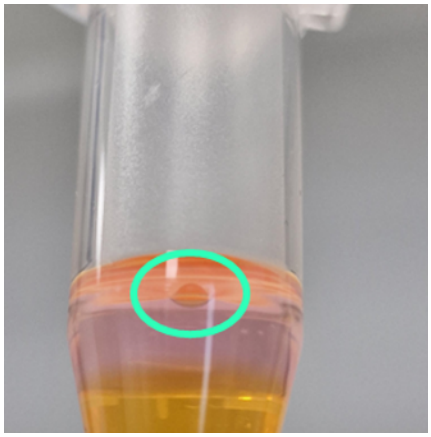
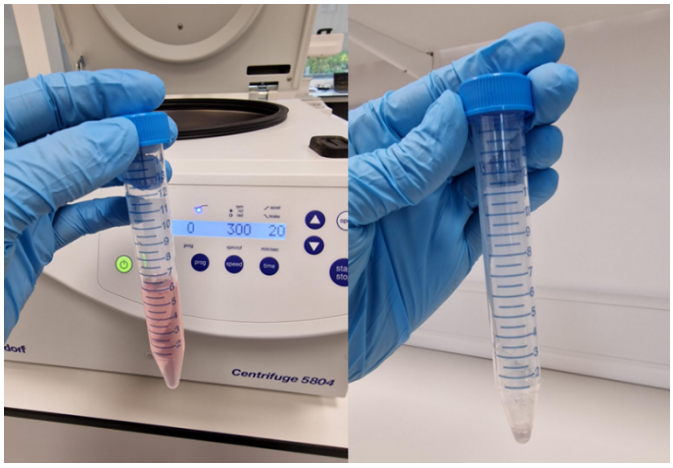
Step	Operation	Details
		• Carefully pipette a layer of 0.5 ml Cyto-Surf® under the media in well A1 as shown below.  •
2	Load the prepared 24-well microtiter plate into the Cyto-Mine® / Cyto-Mine® Chroma	• When prompted by the software, eject the plate stage and load the prepared 24-well microtiter plate. • Complete pooled picodroplets dispensing. For more information, refer to 'Pooled Dispensing' in the Cyto-Mine® and Cyto-Mine® Chroma User Manual. • At the end of the experiment the plate stage will be ejected allowing you to retrieve the 24-well microtiter plate.

3.3 Cell Recovery

Step	Operation	Details
1	Transfer the entire contents of the 24-well microtiter plate to a sterile 5 mL tube	• Once transferred, keep the tube upright and stationary for at least 1 minute, to ensure that the emulsion is floating on the top. ***Note:*** *A 5 mL Eppendorf or similar tube with a tapered base can be used - the smaller the conical base of the tube the better.* ***Note:*** *If the sample is high value and maximum recovery is critical, you can wash the well with a small amount of Cyto-Surf® A and add this to the tube too.*
2	Using a pipette, remove the lower oil phase	• The top phase is the emulsion which contains the dispensed picodroplets, the lower phase is Cyto-Surf® A as shown below. 

		• Before aspirating, when the pipette tip has entered the oil phase, depress the pipette fully to the 2nd stop as shown below. This will blow out any sample in the pipette tip.  **When the tip is in the oil phase, blow out any emulsion before aspirating** • Carefully remove as much of the clear, Cyto-Surf ® A phase as possible without aspirating any of the emulsion. ***Note:*** *To be safe and avoid sample loss, a small amount of oil can be left in the bottom to ensure none of the sample is removed in the process as shown below.* 
3	Add Pico-Break™ to break the emulsion	• Add 0.5 mL Pico-Break™ to the emulsion by positioning the pipette tip against the inner wall of the tube, immediately above the sample interface, avoiding direct sample contact. ***Note:*** *When adding Pico-Break™, put the pipette tip to the wall of the tube above the sample layer to reduce direct contact between Pico-Break™ and the sample as shown below.* • Mix by gently inverting the tube 5x. This is sufficient mixing; no more is needed. The emulsion will start to disperse immediately.

		
4	Allow the mixture to settle for 1 minute to allow the de-emulsification process to complete	• Keep the tube upright whilst allowing the mixture to settle for a maximum of 1 minute. • De-emulsification is complete when the two phases are clearly separated and the top phase containing the cells is no longer cloudy. • **Prolonged exposure to Pico-Break™ can be toxic to cells.** Ensure the cells are not exposed to Pico-Break™ for longer than required, **the maximum recommended time is 1 minute.**
5	Transfer the top sample layer into a 15 mL tube	• Gradually transfer the top de-emulsified sample layer into a 15 mL tube with a 1 mL pipette. Do not transfer any Pico-Break™ as this is toxic to cells with long-term exposure. Pico-Break™ will also pellet down with your cells during centrifugation. ***Note:*** *To be safe and avoid sample contamination, leave a small amount of medium / sample layer at the bottom of the layer (as shown below) to ensure you haven't taken up any Pico-Break™.*  **After adding Pico-Break™ and mixing** **Transfer the sample layer to a 15 mL tube**  **Recovered Sample**  **Residual layer left after sample recovery**

		Note: *Sometimes droplets of Pico-Break™ can float on top of the sample layer as shown below. Gently tap the tube to encourage the droplet to fall.*  • If maximum cell recovery is critical, add 900 µL of fresh culture medium to the remaining sample phase, immediately remove without aspirating any oil, and add to the 15mL tube. Repeat if desired. **Note:** *There is a trade-off between sample recovery and possible long-term cell exposure to Pico-Break™. Take care if adding more media and work as quickly as possible. To maximize outgrowth, we recommend a single transfer only.*
6	Dilute the sample 5-fold with fresh media	• Add 5mL of fresh media into the sample to dilute out the OptiPrep already present. **Note:** *Use fresh medium without Opti-Prep™. OptiPrep™ is a density gradient medium and will interfere with cell sedimentation during centrifugation unless it is sufficiently diluted.*
7	Centrifuge the sample	• Centrifuge at 300 x g for 5 minutes. **Note:** *When pool dispensing a small number of droplets, the pellet may be too small to see easily. Avoid agitating the very bottom of the tube where the cells will have aggregated as shown below.* 
8	Resuspend the cells	• Resuspend cells in an appropriate volume of culture medium • Culture as per standard protocol.

Notes

Notes

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Cyto-Mine® and Cyto-Mine® Chroma: CLASS 1 LASER Product – BS EN / IEC 60825-1:2014

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