TECHNOLOGY NOTE

Protocol for the preparation and use of adherent cells loaded SemaCyte® microcarriers for cell-based assays

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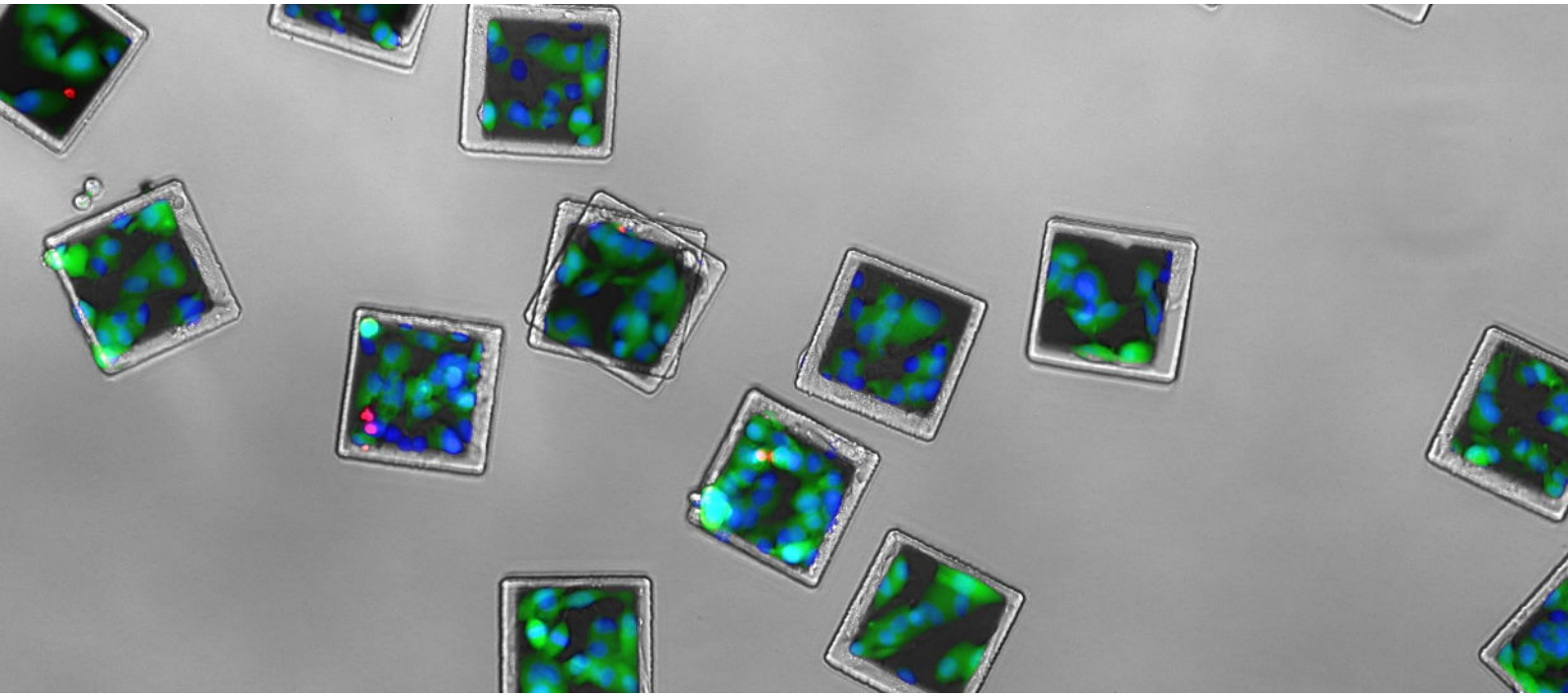
Abstract

Semarion SemaCyte® assaying microcarriers function as ultra-miniaturized wells suspended in liquid. SemaCytes can be compared to mobile islands that carry small colonies of adherent cells and are magnetically steerable. SemaCytes loaded with adherent cells can be frozen and thawed while maintaining cell morphology and cell adhesion during freezing without the need of cell detachment. They can be dispensed with manual or robotic liquid handling tools into multi well plates and are compatible with plate reader and microscopy-based cell assays. In this technology note we present the standard protocol used for the preparation, freezing, and use of SemaCytes loaded with adherent cells.



The SemaCyte® Workflow

Continuous culturing and passaging of cells is a costly operational bottleneck. With the SemaCyte® Seeding Dish it is possible to generate assay-ready adherent cells which can be thawed for instant, on-demand assays. Cells can be seeded on the SemaCyte® Seeding Dish as per standard cell culture protocols. The Seeding Dish contains arrays of immobilised SemaCyte® microcarriers functionalised with bio-polymers to facilitate cell attachment and adhesion. Depending on the cell type used, each SemaCytes can host 5 to 30 cells. Once the right confluency and morphology is achieved, cell loaded SemaCytes are released from the Seeding Dish into suspension. They are subsequently purified and aliquoted into cryovials for cryopreservation and can be stored as assay-ready adherent cells. Before running the assay of choice, SemaCytes can be thawed and dispensed into multi-well plates and used for plate reader assays or imaging immediately after thawing. This approach drastically reduces assay preparation time and resources spent on maintaining cells in culture. The production of batches of assay-ready cells on SemaCytes also reduces experimental variability and increases reproducibility.

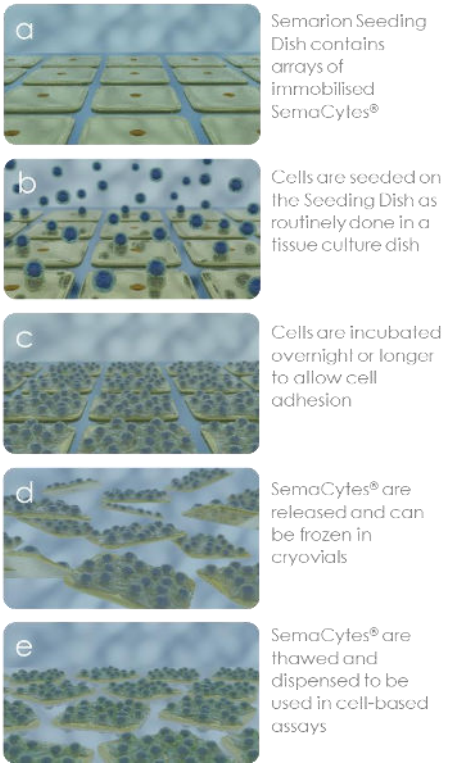


Figure 1. Cell loading and use of Semacytes. *Each key step of the cell loading protocol is shown in the figure. A single cell suspension is prepared and seeded on the Seeding Dish (a-b). Cells are incubated overnight to allow cell adhesion (c) and SemaCytes are then released in suspension and purified on the following day (d), followed by aliquoting and freezing. SemaCytes are thawed on the day of use and dispensed in the plate for the assay of choice (e).*

Required Materials and Reagents

- SemaCyte® Seeding Dish (2-inch, 60 mm)
- SemaCyte® Purification Magnet
- SemaCyte® Dispensing Magnet
- Complete medium (cell type dependent)
- Detaching solution (TrypLE, trypsin, Accutase etc.)
- PBS
- Cryopreservation medium (95% Foetal Bovine Serum, 5% DMSO)
- Cryovials
- Cryovials cooling container (1°C/minute)
- Multi-well plate
- Multichannel pipette
- Wide bore pipette tips

Protocol Overview

The SemaCyte protocol can be broken down into 4 steps as shown in **Figure 2** and **Table 1** and integrates seamlessly with routine culturing procedures and protocols. A suspension the cell of interest is prepared and seeded onto a SemaCyte® Seeding Dish (Day 0). After incubation for 18-24 hours or more, depending on the cell type, SemaCytes are collected, purified, aliquoted, and frozen (Day 1+). Thanks to the magnetic properties of SemaCytes, collection and purification can be performed using a purification magnet. After thawing, SemaCytes are dispensed into a multi-well plate using a double-faced dispensing magnet which orientates the cells depending on the assay of choice, i.e. either facing up or down (Day of use).

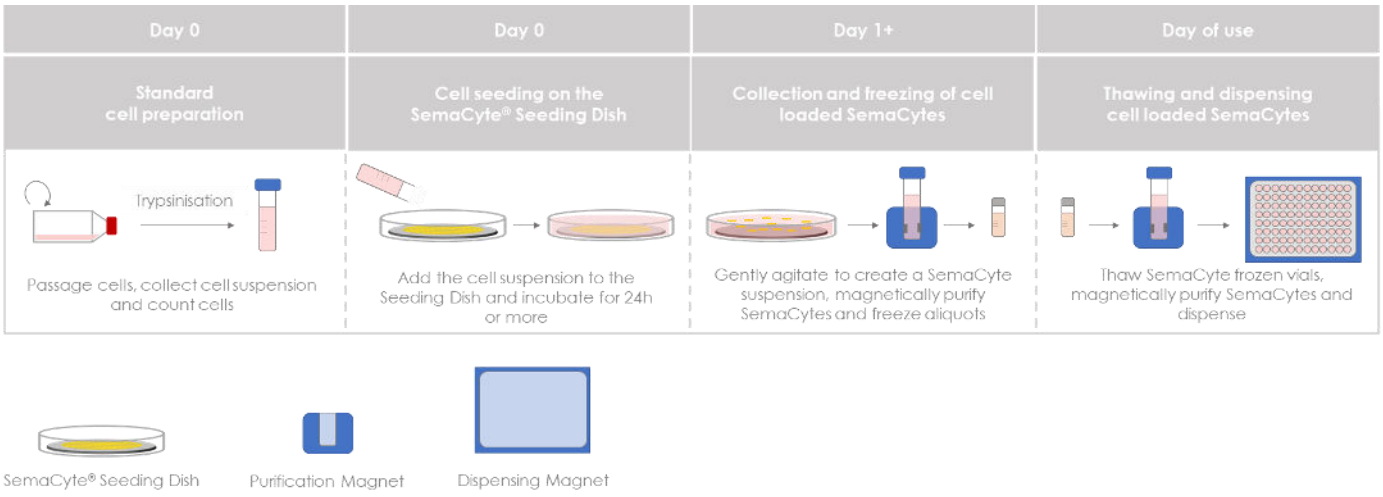


Figure 2. Protocol overview for the preparation and use of adherent cell loaded SemaCyte® microcarriers for cell-based assays. *Each step of the protocol is summarized in the infographic with a brief description of each step. No centrifugation steps are required during at any point in the protocol. A purification magnet is used to collect and purify SemaCytes and a dispensing magnet is used to dispense SemaCytes in the multi-well plate of choice.*

	Steps		Timing	Stop and store
Day 0	1. 2.	Cell preparation Cell seeding on the SemaCyte® Seeding Dish	Dependent on cell type 15 min	N/A
Day 1+	3.	Collection and freezing of cell loaded SemaCytes	45 min	- 80 °C <3 month, -196 °C for longer storage
Day of use	4.	Thawing and dispensing of cell loaded SemaCytes	35 min	N/A

Table 1. Summary of protocol steps, storage conditions, and execution time for each step.

Protocol for the generation of adherent assay-ready cells on SemaCytes

A. Cell preparation

1. Passage and culture the cell-type of interest until cells are 70-80% confluent.
2. Aspirate cell medium.
3. Wash cells with Mg/Ca-free PBS.
4. Add a cell detachment solution (TrypLE, Trypsin or Accutase etc.) to detach adherent cells according to the detachment protocol for the cells of interest. [a]
5. Collect cells in complete medium.
6. Centrifuge cells at an appropriate speed for the cell type used (100-300 g for 5 min is usually fine for most cells).
7. Remove supernatant.
8. Resuspend cells in complete medium. [b]
9. Count cells.

Notes

a) Depending on the cell type used, cell preparation may vary slightly. Always make sure to obtain a single cell suspension before seeding cells on SemaCytes.

b) Cells that are prone to create clumps can be pre-filtered using cell strainers before seeding.

B. Cell seeding on the SemaCyte® Seeding Dish

10. Unpack the SemaCyte® Seeding Dish.
11. Dilute enough cells in 5 mL (for 60 mm dish with surface area of 21.5 cm^2) to obtain the desired seeding density. [a]
12. Slowly dispense the cell suspension on the Seeding Dish and **DO NOT** swirl the liquid after adding the cells. This will increase the risk of releasing and moving of SemaCytes.
13. Place the Seeding Dish in the incubator for 18-24 hours or until cells have attached.

Notes

a) The seeding density required to obtain a sub-confluent or confluent layer of cells on SemaCytes may vary between cell types. Smaller cells may require a higher seeding density as opposed to bigger cell types. This should be tested beforehand.

C. Collection and freezing of cell loaded SemaCytes

14. Shake the dish until the SemaCytes are released or use a 1,000 µL pipette to slowly dislodge SemaCytes.
15. Collect the suspension of SemaCytes and gently add it to a 15 mL tube. [a]
16. Swirl the tube to ensure all SemaCytes are in suspension. Place it into the magnetic tube holder (purification magnet). [b]
17. Wait for 10-12 min until all SemaCytes have been fully captured by the magnet. [c]
18. Gently remove medium from the top of the tube until 1 mL is left. [c]
19. Remove the tube from the magnet. [D]
20. Add 3 mL of fresh medium, swirl the tube to resuspend SemaCytes, and place the tube back into the magnetic tube holder.
21. Wait for 1-2 min until all SemaCytes have been fully captured by the magnet. [c]
22. Gently remove 3 mL of medium from the bottom of the tube. [c]
23. Repeat steps 18-22 once (or twice) more.

Notes

a) If there are still SemaCytes in the dish, an additional wash with 1-2 mL of medium can be performed to collect the remaining SemaCytes.

b) The purification magnet is used to remove debris and unattached cells to obtain a clean solution of SemaCytes loaded with cells.

c) Do not move, twist, or turn the tube during the purification process. Take care not to touch the SemaCyte pellet with the pipette. Make sure that the liquid level does not drop below the magnet.

d) Do not twist or turn the tube while removing it from the magnet.

24. Remove the tube from the magnet and swirl to resuspend SemaCytes. [D]
25. Dilute the SemaCytes and aliquot them into cryovials. [e]
26. Place the cryovial into tube rack for 1-3 min, allowing the SemaCytes to settle by gravity.
27. Remove medium until ~100 µL or less is left.
28. Add ~400 µL of cryopreservation medium to each vial.
29. Store by freezing to -80°C at a rate of 1°C/min.

e) On Seeding Dish contains 50,000 SemaCytes. For well-plate assays, they can be dispensed at 90-1,500 SemaCytes/cm^2. One Seeding Dish thus yields 18-1 cryovials for 96-well plates or 25-1.5 cryovials for 384-well plates.

D. Thawing and dispensing of cell loaded SemaCytes

30. Snap thaw SemaCytes in a 37°C water bath. **DO NOT** swirl the cryovial when thawing.
31. Transfer the contents of the cryovial into a 15 mL tube with 10 mL of fresh medium; a 1/20 dilution. [a]
32. Gently invert twice and place it in the purification magnet. Incubate at room temperature for 10-12 min until all SemaCytes have been fully captured by the magnet. [b]
33. Remove the supernatant from the top until 1 ml of media is left. [b]
34. Remove the tube from the magnet and swirl the tube so that the SemaCytes are resuspended. [b]
35. Dilute the SemaCytes with fresh medium to achieve the desired concentration and volume. [c]
36. Place the well plate onto the dispensing magnet. Confirm that the "dispensing" side faces up. [D]
37. Dispense the SemaCytes, preferably with a multichannel pipette from a liquid reservoir. To ensure homogenous distribution, move the reservoir laterally 5 times between each aspiration. [E]
38. Remove the well plate from the magnetic holder. **DO NOT** shake the well plate again. [F]
39. Incubate at 37°C for 30 min.
40. Perform the assay of interest.

Notes

a) If cells are particularly sensitive to DMSO or a freeze-thaw cycle, it is better to remove most of the cryopreservation medium before transferring it to the tube, further diluting the DMSO.

b) As before, be careful not to disturb the pellet or twist and turn the tube while it is inside the purification magnet.

c) We recommend dispensing 100 µL for 96-well plates and 25 µL for 384-well plates.

d) "Dispensing" side of the magnetic plate holder ensures that the cells are dispensed faced up.

e) It is recommended to use wide-bore pipette tips as cell membranes are sensitive post thawing from DMSO.

f) Performing an additional shaking protocol, such as a figure-eight or cross-like movement, might undo step 36-37.

Additional suggestions

(1) The amount of SemaCytes/cm^2 for a specific assay might depend in the type of endpoint and the incubation time. For example, CellTiter-Glo™ assays 24-72 hours after dispensing can be performed with as little as 90 SemaCytes/cm^2.

(2) The exact concentration of SemaCytes can be determined to adjust concentration and volume in step 35. Add a 10-100 sample µL sample of SemaCytes into a multi-well plate during step 34 and manually count the number of SemaCytes to determine the exact concentration.

(3) The dispensing magnet can be used to flip the SemaCytes for imaging. Place a microwell plate on the dispensing magnet and ensure that the "imaging" side is faced up. This will flip over the SemaCytes so that the cells are towards the objective of inverted microscopes. Agitation or pipetting could can improve the flipping, depending on the type of plate, cell, and assay.

(4) The cell health, morphology, and confluency can be assessed after SemaCyte collection (step 24) and thawing (step 35) by adding a 10-100 sample µL sample of into a multi-well plate. While cells can be observed in a brightfield microscope, we recommend using a live/dead staining kit (e.g. AM-Calcein and Ethidium homodimer-1) with a nuclear dye to assess cell viability and recovery using a fluorescence microscope (**Figure 3**).

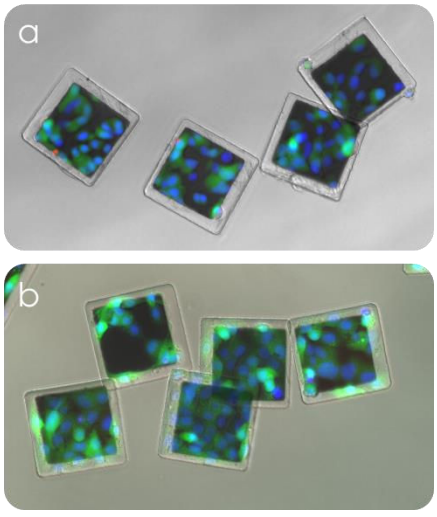


Figure 3. Viability staining on freshly loaded and thawed Semacytes. *HepG2 loaded SemaCytes were purified post-release from the Seeding Dish (a) or post-thawing (b) and stained with AM-Calcein (viable, green), EthD1 (dead, red) and a nuclear dye (blue). The number of viable cells on each SemaCyte can be easily assessed and the quality of loading, or the cell recovery post thawing can be easily calculated.*