SemaCyte® Seeding Dish (SD20)

Basic protocol to generate adherent assay-ready cells on SemaCyte® microcarriers.

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Introduction

SemaCyte® assaying microcarriers function as ultra-miniaturized wells suspended in liquid. SemaCytes loaded with adherent cells can be frozen and thawed while maintaining cell morphology and cell adhesion during freezing. 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. This protocol outlines each of the steps that need to be performed for the preparation, freezing, and use of SemaCyte® assaying microcarriers loaded with adherent cells.

The SemaCyte® Assay-Ready 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 that 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 loading density and cell type used, each standard SemaCyte® can host 4 to 20 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 from 1 hour 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.

The SemaCyte® Seeding Dish 20 (SD20)

SemaCytes are provided as immobilised arrays in a petri dish, called a SemaCyte® Seeding Dish 20. Each dish has a diameter of 60 mm, a total area of 21.5 cm^2, and 50,000 immobilised microcarriers. Each standard SemaCyte® microcarrier has a cell attachment and growth area of 100 µm x 100 µm, or 0.01 mm^2, coated with a

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fibronectin mimetic polymer. They accommodate approximately 4-20 cells, depending on the cell type. Other sizes and coatings are also available.

The recommended density of SemaCytes per well ranges from 40-200 for 96-well microplates and 10-50 for 384-well microplates. Thus, each SD20 contains enough microcarriers for 2-10 microplates worth of experiments.

SemaCyte® Starter Kit

The Seeding Dish 20 (SD20): 50,000 arrayed microcarriers in a 60 mm dish

The SemaPure15: purifies SemaCytes after cell attachment

The SemaPlate: orientates and immobilises SemaCytes inside microplates

Required Materials & Reagents

- SD20 (SemaCyte® Seeding Dish 20)
- SemaPure15 (SemaCyte® Purification Magnet)
- SemaPlate (SemaCyte® Dispensing Magnet)
- Complete medium (cell type dependent)
- Detaching solution (TrypLE™, trypsin, Accutase® etc.)
- Phosphate-buffered saline (PBS)
- Cryopreservation medium (90% Foetal Bovine Serum, 10% DMSO)
- Cryovials
- Cryovial cooling container (1°C/minute)
- Multi-well plates
- Multi-channel pipette
- Wide bore pipette tips

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Protocol Overview

The SemaCyte® protocol can be broken down into 4 steps as shown in **Table 1** and **Figure 1** and integrates seamlessly with routine culturing procedures and protocols. A suspension of the cell type 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+). The magnetic properties of SemaCytes allow for their easy collection and purification 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).

	Steps	Timing	Stop and store
Day 0	1. Cell preparation 2. Cell seeding on the SD20	Dependent on cell type 15 min	N/A
Day 1+	3. Collection and freezing of cell-loaded SemaCytes	45 min	- 80°C <1 month, -196 °C for longer storage
Day of use	4. Thawing and dispensing of cell-loaded SemaCytes	1 h 15 min	N/A

Table 1 *SD20 cell seeding timeline summary.*

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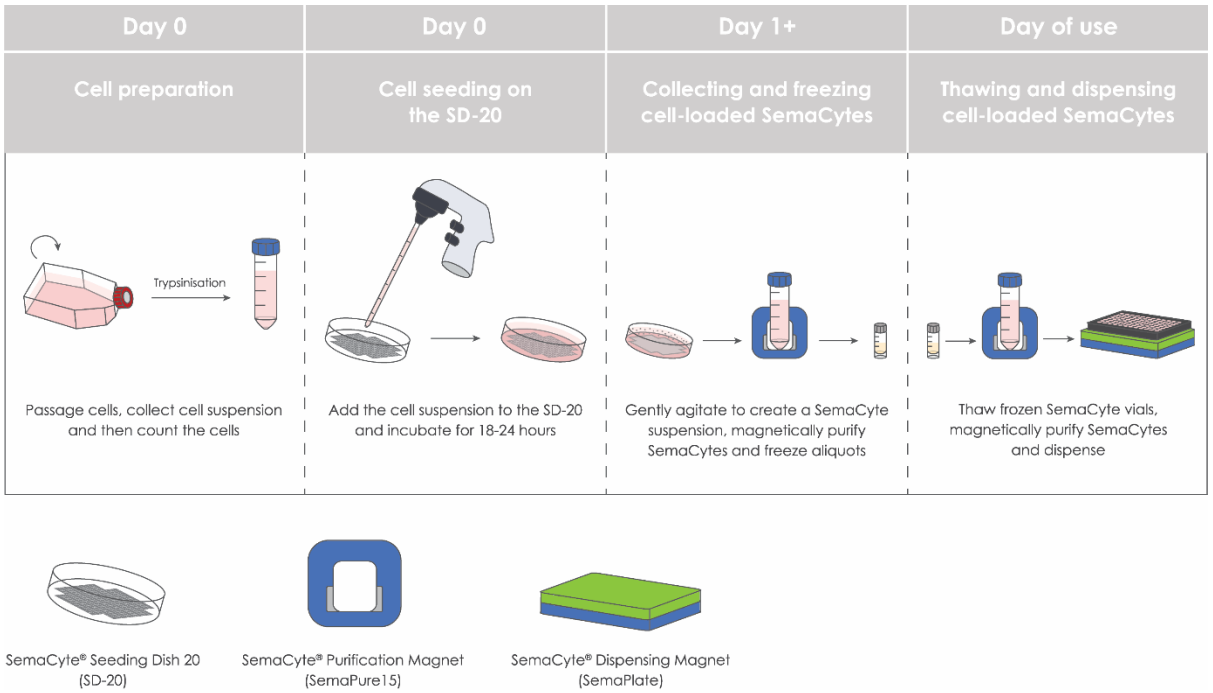

Figure 1 *SD20 cell seeding protocol.*

Protocol for the generation of adherent assay-ready cells on SemaCyte® microcarriers

A. Cell preparation

1. Passage and culture the cell type of interest until cells are ready to be loaded onto SemaCyte® assaying microcarriers.
2. Aspirate cell medium.
3. Wash cells with Mg^{2+}/Ca^{2+} -free PBS.
4. Add a cell detachment solution to detach adherent cells according to the culturing 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).

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7. Remove supernatant.
8. Resuspend cells in complete medium. [b]
9. Count cells.

a) Cell preparation may vary slightly depending on the cell type used. Always make sure to obtain a single cell suspension before seeding cells on SemaCytes.
b) Cells that are prone to clumping can be pre-filtered using cell strainers before seeding.

B. Cell seeding on the SD20

1. Unpack the SD20 and place on the 'cells up' side of the SemaPlate.
2. 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]
3. Slowly dispense the cell suspension onto the SD20. **DO NOT** swirl the liquid after adding the cells, doing so may cause the SemaCytes to release and move. [b]
4. Place the SD20 into the incubator for 18-24 h or until the cells have attached.

a) The seeding density required to obtain a sub-confluent or confluent layer of cells on SemaCytes may vary between cell type, assay duration and format. As a starting point, seeding density for cells on SemaCytes should be similar to that of the same assay on standard plates.
b) Some cells may benefit from leaving the dish in the hood for 1 h before moving it to the incubator. This should facilitate attachment due to less vibration.

C. Collection and freezing of cell-loaded SemaCytes

1. Using a 1,000 µL pipette, gently aspirate media and redispense it over the SemaCytes to dislodge them. This process of pipetting up-and-down can be used multiple times to ensure all SemaCytes are liberated from the surface. Alternatively, SemaCytes can be dislodged by shaking or swirling the dish.
2. Collect the suspension of SemaCytes and gently add it to a 15 mL tube. [a]

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3. Gently invert the tube to ensure all SemaCytes are in suspension and then place it into the SemaPure15 purification magnet. [b]
4. Wait for 5 min until all the SemaCytes have been captured by the SemaPure15. **DO NOT** twist or turn the tube. [c]
5. Gently remove medium from the top of the tube until 1 mL is remaining. [c]
6. Carefully remove the tube from the magnet. **DO NOT** twist or turn the tube. [d]
7. Add 3 mL of fresh medium, invert the tube to resuspend the SemaCytes, and place the tube back into the SemaPure15.
8. Wait for 1-2 min until all SemaCytes have been fully captured by the SemaPure15. **DO NOT** twist or turn the tube. [c]
9. Gently remove 3 mL of medium from the bottom of the tube. [c]
10. Repeat steps 7-9 once (or twice) more.
11. Carefully remove the tube from the SemaPure15. **DO NOT** twist or turn the tube. [d]
12. Allow the SemaCytes to settle to the bottom of the tube.
13. Carefully remove as much medium as possible without disturbing the SemaCytes.
14. Determine the number of cryovials to be prepared and for each vial add 500 µL of cryopreservation medium to the tube containing SemaCytes. [e]
15. Gently resuspend the SemaCytes in cryopreservation medium using a stripette (2-3 ×) and aliquot 500 µL of SemaCytes into each cryovial. [f]
16. Store by freezing to -80°C at a rate of 1°C/min.

a) If there are still SemaCytes in the dish, an additional wash with 1-2 mL of fresh medium can be performed to collect the remaining SemaCytes.
b) The SemaPure15 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 SemaPure15. Pull the tube out with a straight vertical motion.
e) One SD20 contains 50,000 SemaCytes. Vials can be made according to the required number of SemaCytes needed post-thawing.

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f) Gentle inversion of the tube may be required to prevent the SemaCytes from settling whilst aliquoting into cryovials, ensuring an accurate distribution.

D. Thawing and dispensing of cell-loaded SemaCytes

1. Snap thaw SemaCytes in a 37°C water bath (~90 seconds for 500 µL freezing media). **DO NOT** swirl the cryovial when thawing.
2. Remove most of the freezing medium without disturbing the SemaCyte pellet.
3. Slowly add 1 mL of room temperature medium dropwise to the cryovial containing SemaCytes. [a]
4. Transfer the SemaCytes to a 15 mL tube.
5. Wash the cryovial with 1 mL medium then transfer the contents to the tube containing SemaCytes.
6. Add 3 mL medium to the tube (now 5 mL total) and then place the tube into the incubator to allow the cells to recover for 1 h.
7. After 1 h, resuspend the SemaCytes by gently inverting the tube and then place it into the SemaPure15 purification magnet. **DO NOT** twist or turn the tube. Incubate at room temperature for 3-5 min until all SemaCytes have been fully captured by the magnet. [b]
8. Remove the supernatant from the top until 1 mL of medium is remaining and then remove the tube from the magnet. **DO NOT** twist or turn the tube. [b]
9. Dilute the SemaCytes with fresh medium to achieve the desired concentration and volume. [c]
10. Place the well plate required for the assay of choice onto the SemaPlate, ensuring that the "cells up" side faces up. [d]
11. Dispense the SemaCytes into wells. [e]
12. Remove the well plate from the SemaPlate. **DO NOT** shake or swirl the well plate again.
13. Perform the assay of interest.

a) The medium should be dispensed slowly over ~60 sec to reduce osmotic shock to the cells.

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b) Be careful to not disturb the pellet or twist and turn the tube while it is inside the SemaPure15. Make sure that SemaCytes have not deposited below the magnet.
c) We recommend dispensing 100 µL for 96-well plates and 25-50 µL for 384-well plates.
d) The "Cells up" side of the SemaPlate ensures that the cells are dispensed facing up.
e) If using a multi-channel pipette for dispensing, move the dispensing reservoir laterally 5 times between each aspiration. This will ensure homogenous distribution of SemaCytes across the plate.

E. Additional suggestions

1. The amount of SemaCytes/cm^2 for a specific assay may depend on 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 by adding a 10-100µL sample of SemaCytes into a multi-well plate and manually counting the number of SemaCytes.
3. The cell health, morphology, and confluency can be assessed after SemaCyte collection and thawing by adding a 10-100 µL sample of cell-loaded SemaCyte® microcarriers 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.

For additional information and advice, please contact info@semarion.com. We provide guidance for specific assays or tools.

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