SIGMA-ALDRICH®
sigma-aldrich.com
3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Cell-in-a-Box®

Catalog Number **CIB001**
Storage at Room Temperature

CELL IN A BOX
POWERED BY AUSTRIANOVA
YOUR CELLS, OUR DELIVERY

TECHNICAL BULLETIN

Product Description
Research on cell therapy has grown tremendously in recent years, and many diverse applications have been developed utilizing both eukaryotic (e.g., stem cells and therapeutic cell lines for biomedicine, hybridomas for antibody production) and prokaryotic cells (e.g., neutraceuticals).

Cell encapsulation technology has emerged as a very promising platform in this field, synergizing with basic cell therapy approaches to offer enhanced capabilities, improved bioreactor efficiencies, biomolecule production as well as selective and targeted drug delivery systems.

Currently, cell encapsulation is undertaken using large, expensive, and technologically challenging machinery, which is very rarely available to the average scientist in a research setting, and far too expensive to purchase for initial experimentation. This has been the status of the encapsulation industry for the past 30 years, which this novel, manual encapsulation kit aims to change.

This kit is based on proprietary encapsulation materials and methods that can be performed manually by any end user using a simple disposable system. The components are non-toxic and biocompatible. The capsules entrap the cells securely within the lumen of the capsule, yet allow the cells to continue growing within the confined boundaries. Nutrients and secreted factors are able to diffuse and exchange through the membrane pores, which can be selectively tuned.

Components

Solution 1 (Catalog Number S2077)	5 ml
10× Solution 2 (Catalog Number S2202)	50 ml
Sterile 1 ml Luer lock syringes (Catalog Number S2327)	5 each
Sterile blunt-end needles (green plastic) G34, droplet needles (Catalog Number N5290)	5 each
Sterile blunt-end needles (red plastic) G18½, filling needles (Catalog Number N5165)	5 each

Reagents and Equipment Required but Not Provided.
- 250 ml beaker (sterile) with magnetic stir bar
- Magnetic stirrer plate
- Sterile PBS without Ca^{2+} or Mg^{2+} (≥500 ml)
- Sterile water (≥100 ml)
- Electronic timer
- Microcentrifuge tubes and conical tubes
- Serological pipettes (2, 5, 10, 25, and 50 ml) with autopipettor
- Pipette and sterile filtered pipette tips (1 ml)
- Sterile culture flasks or plates
- Optional – spatula for capsule handling
- Optional – Luer lock syringes with other volumes

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. The capsules are not suitable for *in vivo* use; they should not be used for any living animal or human application, whether it be a medical, research, or other. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
Store the kit at room temperature. **Do not freeze.**

Unused Solution 1 and Solution 2 may be stored at room temperature for future use.

Procedure

Most cell types are suitable for encapsulation. Adherent cells are detached from the cell culture dish according to standard cell culture protocol and harvested by centrifugation, while suspension cells can be centrifuged directly. Starting cell densities of 5×10^5 to 5×10^6 cells per ml of Solution 1 are suitable for encapsulation. As a guideline, a density of 2×10^6 cells per ml of Solution 1 is recommended as a starting point and used in this procedure. The density can be adjusted depending on the cell type (see Appendix). This kit contains 5 ml of Solution 1, sufficient for 5 individual encapsulation experiments. The minimal amount recommended per encapsulation is 1 ml, which will yield up to 120 capsules. Capsule yield is dependent on the rate of droplet release, not the volume of Solution 1 used, due to the 1 minute limit on droplet release.

Cells are mixed with Solution 1 and added drop-wise with constant stirring into Solution 2, which acts as the hardening bath. After the droplets have hardened into capsules, a series of wash steps is performed before the encapsulated cells are transferred into growth medium. Most cells will cluster or proliferate to colonize the inside of the capsules and in many cases fill them up (see Results, Figure 1). Growth can be monitored by conventional metabolic activity assays, such as resazurin.

Notes: Ensure all the required items have been prepared beforehand (see Reagents and Equipment Required but Not Provided). All items should be autoclaved and sterile where possible, and all media warmed to room temperature before use.

Always work in the laminar flow hood under sterile conditions using aseptic techniques.

1. Set up the stirring plate and beaker in the laminar flow hood.

2. First, pipette 90 ml of water into the 250 ml beaker. Then, add 10 ml of Solution 2. Rinse the pipette in the hardening bath. Stir for 10 minutes. For encapsulation, reduce the speed of the stir bar to the lowest *practical* stirring speed.

3. Prepare a single cell suspension of cells according to your laboratory procedure (alternatively, use the one outlined in the Appendix).

4. Wash the cells twice in PBS and count them. Take 2×10^6 cells and place them in a sterile 1.5 ml microcentrifuge tube. Pellet them by centrifugation for 5 minutes and discard the supernatant.

5. Add 1 ml of Solution 1 to the cell pellet and resuspend by pipetting up and down until the cells are uniformly dispersed. Avoid formation of air bubbles.

6. Attach the red plastic filling needle (G18½, blunt-end) to the 1 ml Luer lock syringe and draw up the cell suspension. Do not generate air bubbles.

7. Replace the filling needle with the green plastic droplet needle (G34, blunt-end) taking especial care that the needle is screwed firmly in place. Eliminate air bubbles from the syringe. Discard the first few droplets in order to force any air from the needle.

8. Start the timer. Hold the needle vertically, 2–3 cm above the hardening bath. The needle tip must not touch the liquid in the bath. Dispense droplets at a moderate speed of 1–2 drops per second, maintaining the same drop height. Move the needle around slightly to prevent droplets from landing at the same spot in the bath.

9. Continue making as many capsules as required but **do not dispense droplets after 1 minute**.
   Note: If more capsules are desired, repeat the procedure again, with the remaining cell suspension and a fresh hardening bath.

10. After dispensing the last droplet, stir the capsules for 5 minutes. Adjust the stirrer speed to ensure the capsules are moving continuously in the bath.

11. Stop the stirrer and allow capsules to settle. Discard 50 ml of the bath solution using a serological pipette, then dispense 100 ml of sterile PBS into the beaker.

12. Restart the stirring to wash the capsules for 10 minutes.

13. Discard 100 ml of the bath solution and add 100 ml of fresh sterile PBS into the beaker. Wash again for 5 minutes.

14. Discard remaining PBS, leaving just enough liquid to cover all the capsules.

15. Rinse 3 times with 30 ml of PBS, and then 3 additional times with 30 ml of cell culture medium. A rinse cycle is the addition of 30 ml of liquid and then removing it by pipette.

16. Pick up the capsules with a 25 ml serological pipette. Place them in the container or flask of choice. Add an appropriate volume of cell culture medium for maintenance of the encapsulated cells.

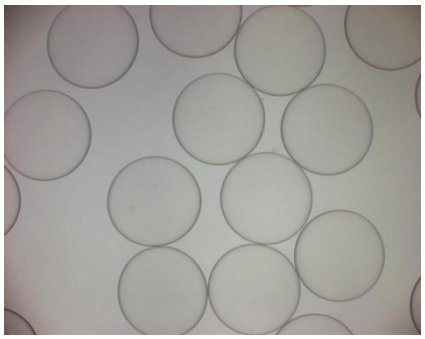
17. Culture the flask in a CO_2 incubator and change the medium at regular intervals (e.g. 2–3 times a week) to keep the encapsulated cells viable.

Notes: To make more capsules from any cell-Solution 1 suspension remaining in the syringe, prepare a fresh hardening bath as described in step 2, and repeat the procedure from step 8 onwards.

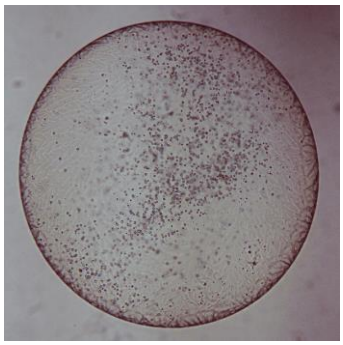
Discard used needles carefully after use.

Results

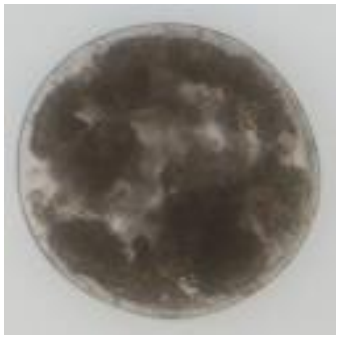
Figure 1.
Encapsulated cells



Empty capsules



Capsule with cells inside directly after encapsulation



Capsule after culturing for days or weeks (depending on cell type and cell growth speed)

Appendices

Calculations and adjustment of encapsulation parameters

1. Use the following equation to calculate the necessary quantities:

$$\mathbf{V_{Sol1}} = \frac{\text{(Total no. of cells)}}{(\mathbf{C_{cell\text{-}Sol1}})} = \frac{(\mathbf{C_{cell\text{-}PBS}}) \times (\mathbf{V_{cell\text{-}PBS}})}{(\mathbf{C_{cell\text{-}Sol1}})}$$

   where:
   $\mathbf{V_{Sol1}}$ = Volume of Solution 1 used to resuspend the cells and to make droplets with
   $\mathbf{V_{cell\text{-}PBS}}$ = Volume of cell suspension in PBS needed for the encapsulation procedure
   $\mathbf{C_{cell\text{-}Sol1}}$ = Desired starting cell encapsulation concentration in Solution 1
   $\mathbf{C_{cell\text{-}PBS}}$ = Empirically calculated cell concentration of the cell-PBS suspension

2. Choose a desired encapsulation density ($\mathbf{C_{cell\text{-}Sol1}}$) for the cells. Starting cell densities of 5×10^5 to 5×10^6 cells per ml of Solution 1 are suitable for encapsulation. A starting density of 2×10^6 cells per ml of Solution 1 is recommended and used in the procedure.

3. Choose an appropriate volume ($\mathbf{V_{Sol1}}$) of Solution 1 to use. For most cases the recommended minimal volume of 1 ml will generate ~150 capsules. Adjust the volume accordingly for more or less capsules.

4. Take the desired volume of cells ($\mathbf{V_{cell\text{-}PBS}}$) from the cell-PBS suspension and pellet to remove the supernatant.

5. Take the required cell-PBS suspension ($\mathbf{V_{cell\text{-}PBS}}$) and pellet it by centrifugating at 200–500 × *g* for 5 minutes. Discard the PBS supernatant.

6. Loosen the cell pellet, then add the required amount of Solution 1 ($\mathbf{V_{cell\text{-}Sol1}}$) to it. Continue with the Procedure, step 7.

Example 1:
If one has a viable cell density ($\mathbf{C_{cell\text{-}PBS}}$) of 1.60×10^6 cells/ml and has 10 ml of cell-PBS suspension, and one wants to use 1 ml of Sol 1 ($\mathbf{V_{Sol1}}$) to encapsulate cells at 2×10^6 cells/ml ($\mathbf{C_{cell\text{-}Sol1}}$), then:

$$1 = \frac{(1.6 \times 10^6) \times (\mathbf{V_{cell\text{-}PBS}})}{(2 \times 10^6)}$$

($\mathbf{V_{cell\text{-}PBS}}$) = 1.25 ml

One takes 1.25 ml of the 10 ml cell-PBS suspension for pelleting.

Example 2:
If one counted a viable cell density ($\mathbf{C_{cell\text{-}PBS}}$) of 0.40×10^6 cells/ml and only have 2 ml of cell-PBS suspension, and one wishes to encapsulate at 1×10^6 cells/ml ($\mathbf{C_{cell\text{-}Sol1}}$), then:

$$1 = \frac{(0.4 \times 10^6) \times (\mathbf{V_{cell\text{-}PBS}})}{(1 \times 10^6)}$$

($\mathbf{V_{cell\text{-}PBS}}$) = 2.5 ml

In this case, one would require 2.5 ml of cell-PBS suspension, but since one only has 2 ml, this is insufficient. Hence, one would have to use all of the cell-PBS solution (2 ml). The quantity of Solution 1 would need to be reduced in order to maintain the same encapsulation concentration ($\mathbf{C_{cell\text{-}Sol1}}$). Hence:

$$(V_{cell\text{-}Sol1}) = \frac{(0.4 \times 10^6) \times (2)}{(1 \times 10^6)} = 0.8 \text{ ml}$$

Use all of the cell-PBS suspension, pellet it to get 0.8×10^6 cells, and resuspend with 0.8 ml of Solution 1.

Generation of single cell suspension and cell counting

1. For adherent cell lines, detach cells by adding 3 ml of trypsin solution to a single T175 flask of confluent cells. Then add 7 ml of cell medium with 10% FBS or equivalent to deactivate the trypsin. Pipette up and down to break up the cell clumps.

2. For suspension cell lines, proceed with step 3.

3. Transfer the single cell suspension into a 50 ml centrifuge tube and the pellet the cells by centrifugation at 200–500 × *g* (cell-specific) for 5 minutes.

4. Discard the supernatant and loosen the cell pellet by gently shaking the tube.

5. Resuspend the cells in 40 ml of PBS for washing.

6. Pellet the cells again at 200–500 × *g* for 5 minutes and resuspend again in 10–40 ml of PBS [$\mathbf{V_{cell\text{-}PBS}}$] (*to adjust cell concentration during counting*).

7. Count the number of cells in the suspension using a standard laboratory method (e.g. hemocytometer, Trypan blue staining is optional.)

Troubleshooting Guide

Capsules do not sink into hardening bath after dropping.	Falling capsules do not have enough impact energy to break through the surface of the bath. Increase the distance between the needle tip and the bath surface.
Capsules stick to each other	Capsules are colliding with each other in their early phase of hardening. Move the needle around slightly so that each droplet does not land in the same spot as the previous one. If the problem is related to having too many capsules in the hardening bath, reduce number of capsules made.
Capsules form with a bubble inside	a) Capsules are hitting the bath surface too hard, creating a 'backsplash' effect that results in a bubble being trapped inside the droplet when it hardens. Lower the drop height of the needle. b) Too many bubbles in cell suspension. See below.
Capsules are deformed or elongated	Stirring speed is set too fast during hardening phase, causing the droplet to be deformed while hardening. Reduce stirrer speed.
Capsules are crumpled or collapsed	Capsule has exceeded recommended hardening time. Reduce the hardening time.
Cell-Solution 1 suspension has too many mini bubbles	Centrifuge the Cell-Sol1 suspension for 5 minutes at 200 × *g* to get rid of the bubbles, and then resuspend the cells again more carefully.
Cells are seen outside of the capsules during culture	**Possible causes:** a.) Capsules may not have formed properly, or may have ruptured during handling, allowing cells to escape. b.) Cell clumps were embedded in the capsule membrane, shielded from the toxicity of Solution 2 during hardening, and managed to subsequently escape during culture. **Solutions:** a.) Check for holes and tears in the capsule. Discard damaged capsules. Wash intact capsules thoroughly in PBS/culture medium and transfer to a clean flask. In most cases this step should be enough. b.) If external cell proliferation persists, incubate intact capsules normally, in cell culture medium supplemented with a 2,500× dilution of Solution 2 (10×) stock solution and observe. External cells should die within 1 day. Otherwise, carefully increase the Solution 2 concentration in the culture medium until it is toxic to the cells. It should not be necessary to go beyond a 250× dilution. Capsule stickiness might be observed in some cases; therefore, the user may choose to culture the capsules at low density or individually. c.) Encapsulation can be repeated at a lower cell density, which significantly reduces the chance of cells embedding on the capsule membrane.
Capsules surface looks lumpy and has irregular patches after encapsulation	Cell encapsulation density might be too high. Encapsulation can be repeated at a lower cell density, which significantly reduces the irregularity of the capsule formation process

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