CS-SNS

Preservation of Cells and Viruses in Suspension

Table of Contents

1 Components

1.1 Kit Contents

Product Code	Components	Units	Unit Volume	Cell Suspension to Add
CS-SNS-03	CytoStor Vial (beads)	3 tubes	0.4 mL	-
	Gel A (5x)	3 tubes	0.12 mL	0.48 mL
	Dissolution Buffer	3 tubes	1.1 mL	-
CS-SNS-06	CytoStor Vial (beads)	6 tubes	0.4 mL	-
	Gel A (5x)	6 tubes	0.12 mL	0.48 mL
	Dissolution Buffer	6 tubes	1.1 mL	-
CS-SNS-12	CytoStor Vial (beads)	12 tubes	0.4 mL	-
	Gel A (5x)	12 tubes	0.12 mL	0.48 mL
	Dissolution Buffer	12 tubes	1.1 mL	-
CS-SNS-24	CytoStor Vial (beads)	24 tubes	0.4 mL	-
	Gel A (5x)	24 tubes	0.12 mL	0.48 mL
	Dissolution Buffer	24 tubes	1.1 mL	-
CS-SNS-50	CytoStor Vial (beads)	50 tubes	0.4 mL	-
	Gel A (5x)	50 tubes	0.12 mL	0.48 mL
	Dissolution Buffer	50 tubes	1.1 mL	-

NOTE: Remove components from 2-8°C storage for at least 20 minutes before use

1.2 Components to be Supplied by the User

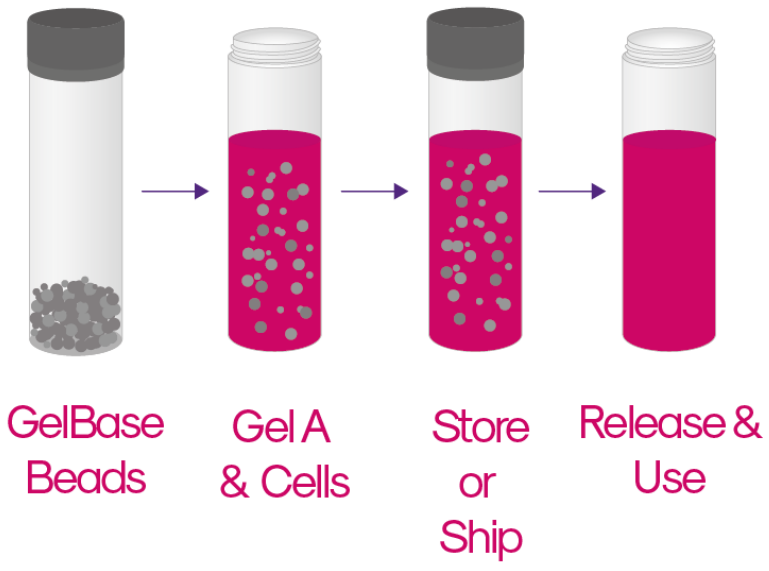
1000 µL pipettes and tips

Cell culture medium

1.3 Before You Begin using CytoStor™

1. If you have not already, we recommend you fill in our Technical Support Questionnaire to get tailored support for your cell type. Fill in the questionnaire at https://www.atelerix.co.uk/technical-support-questionnaire/.
2. Ensure CytoStor™ kits have not passed the expiry date stated on the packaging. Atelerix does not recommend using kits after this date.
3. Read the troubleshooting guide on page 9 to see our list of frequently asked questions. For any further queries, please email us at technical@atelerix.co.uk.
4. Consult the Cell Density and Loading guide (page 4 for cells, page 7 for viruses) and the Cell and Virus Storage Temperature Guide on page 10.
5. CytoStor™ is intended for use solely in accordance with this protocol using the components provided within the kit.

2 Protocol Overview



3 CytoStor™ for Encapsulation of Cells

3.1 Gelation

1. Ensure that all components are allowed to equilibrate to room temperature before use and that gels are at the bottom of their tubes. Conduct all steps in a laminar flow hood at room temperature.
2. Resuspend cells in the appropriate volume of culture medium (See 3.2 Cell Density and Loading Guide on page 4 for more information), ensuring thorough distribution of cells.
3. Add 0.48 mL of the cell culture suspension to the vial containing 0.12 mL of Gel A.
4. Gently mix until homogenous, with a pipette, ensuring that no bubbles are introduced (see troubleshooting guide on page 9).
5. Add 0.6 mL of the cells / Gel A mix to the CytoStor Vial[1].
6. Place the cap back on the tube and gently invert the **gel / bead** mixture several times until the beads are evenly distributed throughout the gel. Gently flick the tube to settle the contents, ensuring a tight seal (the gel will cure *in situ* within approximately 30 minutes, sample is ready to ship after 1 hour).
7. Store away from light in a polystyrene box at the recommended temperature for the cell type encapsulated. See the table on page 10 or, for the most up to date recommendations on storage temperatures and times, please check our dedicated webpage by visiting https://www.atelerix.co.uk/guidelines-for-testing-conditions/ or by scanning the QR code.

[1]Use the CytoStor Vial containing beads provided for encapsulation, storage, and release.

3.2 Cell Density and Loading Guide

Cell loading guide

Desired cell load per sample ($x 10^6$)	Cell suspension concentration ($x 10^6$ cells/mL)	Volume of cell suspension per sample (mL)
1	2.08	0.48
2	4.17	0.48
3	6.25	0.48
4	8.33	0.48
5	10.42	0.48
6	12.50	0.48
7	14.58	0.48
8	16.67	0.48
9	18.75	0.48
10	20.83	0.48

For multiple encapsulations, multiply the volume of cell suspension (C) by the number of samples required.

Recommended Cell Load for different cell types

Cell Diameter	Example Cell Types	Recommended Cell Load per Encapsulation
4-10 Microns	Lymphocytes e.g., T cells, PBMCs	2 x 10^7 cells
11-15 Microns	Fibroblasts CHO cells HEK cells	≤ 1 x 10^7 cells
16-30 Microns	Mesenchymal stem cells Monocytes Hepatocytes	≤ 1 x 10^7 cells

CytoStor™ has no lower limit to the number of cells which can be stored, however we recommend using enough cells to be easily recovered after centrifugation.

3.3 Release

1. Ensure that all components and samples are allowed to equilibrate to room temperature before use and conduct all steps in a laminar flow hood at room temperature.
2. Using a pipette tip or syringe and needle, infuse 1 mL Dissolution Buffer into the bottom of the gel by piercing the gel.
3. Place the cap back on the tube and allow the gel to dissolve by occasionally agitating the tube by gentle inversion or rocking for **10 minutes** (see troubleshooting guide on page 9).
4. Sediment cells by centrifugation at 350RCF for **5 minutes**, remove supernatant, and re-suspend cells in medium of choice.

3.4 Shipping Your Cells

Use appropriate controlled room temperature packaging[2] when preparing cells for shipping to reduce the effect of ambient temperature change on the encapsulated cells during transit.

[2]For best cell recovery upon arrival, we recommend using the CoolGuard™ Advance CRT container. Find out more at https://pelibiothermal.com/products/coolguard-advance.

4 CytoStor™ for Encapsulation of Viruses

4.1 Gelation

1. Ensure that all components are allowed to equilibrate to room temperature before use and that gels are at the bottom of their tubes. Conduct all steps in a laminar flow hood at room temperature.
2. Resuspend virus in the appropriate volume of medium (See 4.2 Viral Titre and Loading Guide on page 7 for more information), ensuring thorough distribution of virus.
3. Add 0.48 mL of the virus suspension to the vial containing 0.12 mL of Gel A.
4. Gently mix until homogenous, with a pipette, ensuring that no bubbles are introduced (see troubleshooting guide on page 9).
5. Add 0.6 mL of the virus / Gel A mix to the CytoStor Vial[1].
6. Place the cap back on the tube and gently invert the **gel / bead** mixture several times until the beads are evenly distributed throughout the gel. Gently flick the tube to settle the contents, ensuring a tight seal (the gel will cure *in situ* within approximately 30 minutes, sample is ready to ship after 1 hour).
7. Store away from light in a polystyrene box at the room temperature (15°C - 25°C).

[1]Use the CytoStor Vial containing beads provided for encapsulation, storage, and release.

4.2 Viral Titre and Loading Guide

Loading guide

Desired viral load per sample (x 10^8)	Virus Titre (x 10^8 IFU/mL)	Volume of virus suspension per sample (mL)
1	2.08	0.48
2	4.17	0.48
3	6.25	0.48
4	8.33	0.48
5	10.42	0.48
6	12.50	0.48
7	14.58	0.48
8	16.67	0.48
9	18.75	0.48
10	20.83	0.48

For multiple encapsulations, multiply the volume of virus suspension (C) by the number of samples required.

Recommended Viral Load

Sample	Example Types	Recommended viral Load per Encapsulation
Virus	Lentivirus, Coronavirus, viral vectors	1 x 10^9 IFU

CytoStor™ has no lower limit to the amount of virus which can be stored, however we recommend using enough to be easily recovered after centrifugation.

4.3 Release

1. Ensure that all components and samples are allowed to equilibrate to room temperature before use and conduct all steps in a laminar flow hood at room temperature.
2. Using a pipette tip or syringe and needle, infuse 1 mL Dissolution Buffer into the bottom of the gel by piercing the gel.
3. Place the cap back on the tube and allow the gel to dissolve by occasionally agitating the tube by gentle inversion or rocking for 10 minutes (see troubleshooting guide on page 9).
4. Virus can then either be:
    a. Sedimented by ultracentrifugation. Following ultracentrifugation, remove supernatant and re-suspended virus in medium of choice.
    b. Diluted down in medium of choice (minimum 1 in 4 dilution recommended).

4.4 Shipping Your Virus

Use appropriate controlled room temperature packaging[2] when preparing virus for shipping to reduce the effect of ambient temperature change on the encapsulated virus during transit.

[2]For best recovery upon arrival, we recommend using the CoolGuard™ Advance CRT container. Find out more at https://pelibiothermal.com/products/coolguard-advance.

5 Troubleshooting Guide

Problem / Question	Guidance
I have air bubbles in the gel after mixing with my media, is this a problem?	Air trapped within the gel will affect preservation, so bubbles should be eliminated before mixing with the beads. Allow time for the mixture to settle and the bubbles to travel to the surface before addition.
Can I use the kit to encapsulate organoids and / or spheroids?	Yes, organoids / spheroids can be encapsulated by following the same protocol. Although for recovery we would recommend washing in the tube by repeatedly allowing the material to settle by gravity and carefully removing the supernatant.
Can I ship the Dissolution Buffer in the same package as the samples?	Yes, the Dissolution Buffer is stable at a wide range of temperatures and can be shipped together with the encapsulated samples.
What are the recommended storage times and temperatures for my cell type?	A guide to the recommended storage times and temperatures can be found on page 10 of this book and at https://www.atelerix.co.uk/guidelines-for-testing-conditions/ If you cannot find any recommendations for your cell type please contact technical@atelerix.co.uk.
Can I reuse the contents of the kit if I don't use it all?	No, there should only be sufficient volume for a set number of encapsulations per kit. Any spare reagents will not be sufficient to perform any additional encapsulations properly.
Can I use PBS instead of media when encapsulating samples?	No, PBS should not be used at any point as it inhibits and slowly reverses gelation.
Can I split the kit into smaller tubes to get more encapsulations?	No, we do not recommend removing the beads from the tubes supplied or deviating from the volumes stated.
What if the beads have not fully dissolved after 10 minutes? Can the cells be allowed to sit in the buffer for longer?	This sometimes occurs when the medium used to store the beads fortifies the gel. Extend the incubation until the beads dissolve - this should not take much longer than 15 minutes. Please contact technical@atelerix.co.uk for information on how long cells may be allowed to incubate in the Dissolution Buffer.

6 Statements

6.1 Kit Storage and Stability

This kit is stable at 2-8°C for 6 months. Atelerix does not recommend using the kit after the expiry date stated on the packaging.

6.2 Cellular Material

Please ensure that cell cultures are free of fungal and bacterial contamination before proceeding.

6.3 Trademarks

CytoStor™ is a trademark of Atelerix Ltd.

7 Appendix

7.1 Cell and Virus Storage Temperature Guide

Cell Type	Recommended Storage Conditions	Tested Storage Time
PBMCs	2 - 8˚C	3 days
Jurkat cells	15 - 25˚C	14 days
MSCs	15 - 20˚C	21 days
Monocytes	2 - 8˚C	5 days
Fibroblasts	15 - 20˚C	14 days
Viruses	15 - 25˚C	14 days

If you cannot find any recommendations for your cell type, please contact technical@atelerix.co.uk.

Notes