BR-MNS

Preservation of Cells Suspended in Gel Beads

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1 Components

1.1 Kit Contents

Product Code	Components	Units	Unit Volume
BR-MNS-03	Component A (2x)	1 tube	1 mL
	Component B	3 tubes	4 mL
	Dissolution Buffer	3 tubes	6 mL
BR-MNS-06	Component A (2x)	1 tube	2 mL
	Component B	6 tubes	4 mL
	Dissolution Buffer	6 tubes	6 mL
BR-MNS-12	Component A (2x)	1 tube	4 mL
	Component B	12 tubes	4 mL
	Dissolution Buffer	12 tubes	6 mL
BR-MNS-24	Component A (2x)	1 tube	8 mL
	Component B	24 tubes	4 mL
	Dissolution Buffer	24 tubes	6 mL
BR-MNS-50	Component A (2x)	2 tubes	8.5 mL
	Component B	50 tubes	4 mL
	Dissolution Buffer	50 tubes	6 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

Sterile microcentrifuge tubes or other suitable tubes

21G needle

1 mL Luer slip syringe

1000 µL and 200 µL pipettes and tips

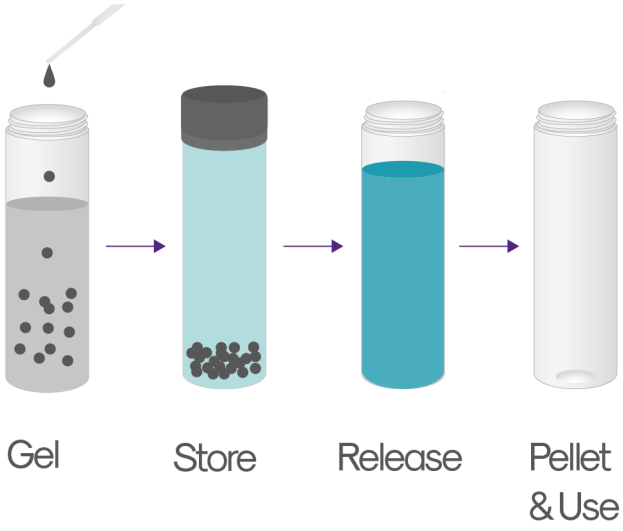
Cell culture medium

1.3 Before You Begin using BeadReady™

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 BeadReady™ kits have not passed the expiry date stated on the packaging. Atelerix does not recommend using kits after this date.
3. Visit https://www.youtube.com/watch?v=HVSDjfmrL-Y and watch our BeadReady™ video protocol.
4. Read the troubleshooting guide on page 7 to see the list of frequently asked questions. For any further queries, please email us at technical@atelerix.co.uk.
5. Consult the Cell Density and Loading guide on page 5 and the Cell Storage Temperature Guide on page 8.
6. BeadReady™ is intended for use solely in accordance with this protocol using the components provided within the kit.

2 Step-by-Step Guide

2.1 Overview



2.2 Gelation

1. Ensure that all components are allowed to equilibrate to room temperature before use and conduct all steps in a laminar flow hood at room temperature.
2. Resuspend cells in the appropriate volume of culture medium (See 2.3 Cell Density and Loading Guide on page 5), ensuring thorough distribution of cells.
3. Add the appropriate volume of Component A to the cell suspension (See 2.3 Cell Density and Loading Guide on page 5), pipetting slowly to ensure the full volume of Component A is drawn.
4. Mix thoroughly but slowly using a pipette 5 – 10 times, or until cells are fully suspended.
5. For each BeadReady encapsulation, draw up the solution into a 1 mL syringe ensuring that no bubbles are introduced into the system (see troubleshooting guide on page 7*). N.B. Before drawing solution, ensure there is an air-space in the syringe to allow full purge of the solution volume when dispensed.*
6. Slowly drop 0.5 mL of the cell/gel solution through a 21G needle into Component B at a height of approximately 1.5 cm above the surface of the liquid to form beads.[1]

7. Once completed, allow beads to stabilize at room temperature for **8 minutes** in Component B.
8. Remove Component B from the gel beads using a 1000 µL pipette or syringe with needle, guiding the tip of the pipette or syringe needle down the inside of the tube to avoid disturbing gelled beads.
9. Wash the beads by adding 1 mL culture medium and leave for **2 minutes**.
10. Remove the washing medium using a pipette without disturbing the beads before replacing with 5.5 mL culture medium for storage.
11. Tightly seal the tube. Store away from light at the recommended temperature for the cell type encapsulated. See the table on page 8 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 collection tube provided containing Component B for encapsulation, storage, and release.

2.3 Cell Density and Loading Guide

Cell loading guide

A Desired cell load per sample (x 10^6)	B Cell suspension concentration (x 10^6 cells/mL)	C Volume of cell suspension per sample (mL)	D Volume of Component A per sample (mL)
1	4	0.275	0.275
2	8	0.275	0.275
3	12	0.275	0.275
4	16	0.275	0.275
5	20	0.275	0.275
6	24	0.275	0.275
7	28	0.275	0.275
8	32	0.275	0.275
9	36	0.275	0.275
10	40	0.275	0.275

For multiple encapsulations, multiply the volume of cell suspension (C) and Component A (D) by the number of samples required. Note: 10% is calculated into all volumes (C&D) to account for error.

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

BeadReady™ 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.

2.4 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. Remove the culture medium from beads. Add 5 mL of Dissolution Buffer, without disturbing the beads.
3. Place the cap back on the tube and allow the beads to fully dissolve by occasionally agitating the tube by gentle inversion or rocking for 10 minutes (see troubleshooting guide on page 7).
4. Sediment the cells by centrifugation at 350RCF for 5 minutes, remove supernatant, and re-suspend cells in medium of choice.

2.5 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.

2.6 Troubleshooting Guide

Problem / Question	Guidance
I have air bubbles in the gel after mixing my cells, is this a problem?	Air trapped within the beads will affect preservation, so bubbles should be eliminated before gelation. Allow time for the mixture to settle and the bubbles to travel to the surface before drawing into the syringe and ensure this is done slowly.
I have a lower than recommended cell count, can I still encapsulate?	Yes, although good recovery cannot be guaranteed if encapsulating fewer cells than is retrievable by centrifugation. If you have any feedback regarding cell density, please contact us at technical@atelerix.co.uk.
Can I ship the Dissolution Buffer in the same package as the cells?	Yes, the Dissolution Buffer is stable at a wide range of temperatures and can be shipped together with the encapsulated cells.
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 8 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.
I don't have access to a syringe and / or needle, can I still use the kit?	Yes, you can use a pipette to drop the gel as an alternative to using a syringe and needle, although we highly recommend using low retention tips. Simply dispense the gel slowly to allow droplets to form and fall individually and allow a few seconds between drops to allow for gelation.
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 storing encapsulated cells?	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 Component B 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.

3 Statements

3.1 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.

3.2 Cellular Material

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

3.3 Trademarks

BeadReady™ is a trademark of Atelerix Ltd.

4 Appendix

4.1 Cell 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

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

Notes