atelerix

WellReady™

WR-N384; WR-N096

In-Plate Preservation of Air-Sensitive Cells for 384- and 96-well plates

Table of Contents

1 Components

1.1 Kit Contents

Product Code	Components	Units	Unit Volume	Medium to Add
WR-N384-03	Gel A (2.5x)	3 tubes	2.4 mL	4.8 mL
	Gel B (5x)	3 tubes	2.4 mL	9.6 mL
	Gel C (5x)	3 tubes	2.4 mL	9.6 mL
	Gelation Buffer 1	2 tubes	15 mL	-
	Gelation Buffer 2	2 tubes	15 mL	-
	Dissolution Buffer	3 tubes	6.5 mL	-
	Adhesive Plate Seals	4	-	-
WR-N384-06	Gel A (2.5x)	6 tubes	2.4 mL	4.8 mL
	Gel B (5x)	6 tubes	2.4 mL	9.6 mL
	Gel C (5x)	6 tubes	2.4 mL	9.6 mL
	Gelation Buffer 1	3 tubes	20 mL	-
	Gelation Buffer 2	3 tubes	20 mL	-
	Dissolution Buffer	6 tubes	6.5 mL	-
	Adhesive Plate Seals	7	-	-
WR-N384-12	Gel A (2.5x)	12 tubes	2.4 mL	4.8 mL
	Gel B (5x)	12 tubes	2.4 mL	9.6 mL
	Gel C (5x)	12 tubes	2.4 mL	9.6 mL
	Gelation Buffer 1	6 tubes	20 mL	-
	Gelation Buffer 2	3 tubes	20 mL	-
	Dissolution Buffer	6 tubes	6.5 mL	-
	Adhesive Plate Seals	7	-	-
WR-N384-24	Gel A (2.5x)	24 tubes	2.4 mL	4.8 mL
	Gel B (5x)	24 tubes	2.4 mL	9.6 mL
	Gel C (5x)	24 tubes	2.4 mL	9.6 mL
	Gelation Buffer 1	12 tubes	20 mL	-
	Gelation Buffer 2	12 tubes	20 mL	-
	Dissolution Buffer	24 tubes	6.5 mL	-
	Adhesive Plate Seals	25	-	-
WR-N384-50	Gel A (2.5x)	50 tubes	2.4 mL	4.8 mL
	Gel B (5x)	50 tubes	2.4 mL	9.6 mL
	Gel C (5x)	50 tubes	2.4 mL	9.6 mL
	Gelation Buffer 1	24 tubes	20 mL	-
	Gelation Buffer 2	24 tubes	20 mL	-
	Dissolution Buffer	50 tubes	6.5 mL	-
	Adhesive Plate Seals	52	-	-

Product Code	Components	Units	Unit Volume	Medium to Add
	Gel A (2.5x)	3 tubes	2.2 mL	3.3 mL
	Gel B (5x)	3 tubes	2.2 mL	8.8 mL
	Gel C (5x)	3 tubes	2.2 mL	8.8 mL
WR-N096-03	Gelation Buffer 1	1 tube	21 mL	-
	Gelation Buffer 2	1 tube	21 mL	-
	Dissolution Buffer	3 tubes	7.5 mL	-
	Adhesive Plate Seals	4	-	-
	Gel A (2.5x)	6 tubes	2.2 mL	3.3 mL
	Gel B (5x)	6 tubes	2.2 mL	8.8 mL
	Gel C (5x)	6 tubes	2.2 mL	8.8 mL
WR-N096-06	Gelation Buffer 1	2 tubes	21 mL	-
	Gelation Buffer 2	2 tubes	21 mL	-
	Dissolution Buffer	6 tubes	7.5 mL	-
	Adhesive Plate Seals	7	-	-
	Gel A (2.5x)	12 tubes	2.2 mL	3.3 mL
	Gel B (5x)	12 tubes	2.2 mL	8.8 mL
	Gel C (5x)	12 tubes	2.2 mL	8.8 mL
WR-N096-12	Gelation Buffer 1	4 tubes	21 mL	-
	Gelation Buffer 2	4 tubes	21 mL	-
	Dissolution Buffer	12 tubes	7.5 mL	-
	Adhesive Plate Seals	13	-	-
	Gel A (2.5x)	24 tubes	2.2 mL	3.3 mL
	Gel B (5x)	24 tubes	2.2 mL	8.8 mL
	Gel C (5x)	24 tubes	2.2 mL	8.8 mL
WR-N096-24	Gelation Buffer 1	8 tubes	21 mL	-
	Gelation Buffer 2	8 tubes	21 mL	-
	Dissolution Buffer	24 tubes	7.5 mL	-
	Adhesive Plate Seals	25	-	-
	Gel A (2.5x)	50 tubes	2.2 mL	3.3 mL
	Gel B (5x)	50 tubes	2.2 mL	8.8 mL
	Gel C (5x)	50 tubes	2.2 mL	8.8 mL
WR-N096-50	Gelation Buffer 1	16 tubes	21 mL	-
	Gelation Buffer 2	16 tubes	21 mL	-
	Dissolution Buffer	50 tubes	7.5 mL	-
	Adhesive Plate Seals	52	-	-

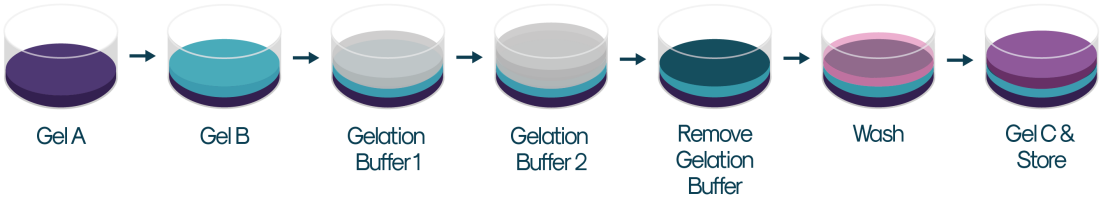
NOTE: Remove components from 2-8°C storage for at least 20 minutes before use.

2 Before You Begin using WellReady™

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 WellReady™ 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=S5Cd8At-tQo and watch our WellReady™ video protocol.
4. Read the troubleshooting guide on page 10 to see the list of frequently asked questions. For any further queries, please email us at technical@atelerix.co.uk.
5. WellReady™ is intended for use solely in accordance with this protocol using the components provided within the kit.

3 Protocol Overview

3.1 Overview



3.2 Cell Confluence

We recommend storing cells at no greater than the preferred confluence, which is usually 70 – 90%.

4 WellReady™ 384-well plates (WR-N0384)

4.1 Components to be Supplied by the User

384-well plate[1] with adherent cell cultures

1000 µL and 200 µL pipettes and tips

Cell culture medium

Multichannel pipette (optional)

Reagent reservoir(s) (optional)

[1]This protocol is intended for use with standard flat bottomed 384 well plates. If your plates are U- or V-bottomed, or will otherwise hold a non-standard volume, please contact technical@atelerix.co.uk for advice on amending the protocol to suit your needs.

4.2 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. Dilute Gel A by adding 4.8mL of complete culture medium and dilute Gel B and Gel C by adding 9.6mL of complete culture medium directly to the tube. Mix until homogenous, either on a vortex for 10 seconds or with a pipette 5 – 10 times, ensuring that no bubbles are introduced (see troubleshooting guide on page 10). *N.B. If mixing with a pipette, take extra care to ensure Gel B is homogenous, as it is much more viscous than Gel A and Gel C.*
3. Carefully remove culture medium from each well of your plate, ensuring that 12.5 µL of culture medium remains in place (*i.e. if the wells contain 50 µL medium, remove 37.5 µL*).
4. Add 12.5 µL of the diluted Gel A solution to each well.
5. Gently add 25 µL of the diluted Gel B solution on top of the Gel A solution.
6. Add 20 µL of Gelation Buffer 1 (GB1) dropwise onto the surface of the Gel A/B solutions. Place the lid back onto the plate and allow 10 minutes for Gelation.

7. Add 20 µL of Gelation Buffer 2 (GB2) dropwise onto the surface of the Gel A/B solutions. Place the lid back onto the plate and allow a further 10 minutes for gelation.
8. Avoiding touching the gel, carefully remove GB1/GB2 mixture from each well and wash for 5 minutes with 50 µL culture medium per well.
9. Carefully remove the culture medium and add 25 µL of the diluted Gel C solution to the centre of each gelled surface.
10. Place an adhesive plate seal over the surface of the plate ensuring it is properly sealed. Place the lid back on the plate and store away from light at the recommended temperature for the cell type encapsulated. See the table below 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.

4.3 Cell Storage Temperature Guide

Cell Type	Recommended Storage Conditions	Testing Time of Encapsulation
iPSC-derived Cortical Neurons	15 – 25˚C	5 days

4.4 Release

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. Remove the plate seal(s) and, by piercing the surface of the gel with the pipette tip, infuse 15 µL of Dissolution Buffer onto the gel within each well of the plate. Use p1000 pipette tip for infusion. Allow **10 minutes** for gel dissolution.
3. Carefully remove 75 µL of the well contents ensuring that you aspirate the liquified gel from the upper part of the well.
4. Add 60 µL of complete culture medium and allow to rest for **15 minutes**.
5. Carefully remove 60 µL of the well contents.
6. Add 30 µL of complete culture medium and return to normal culture conditions for at least **4 hours** or overnight.
7. After the incubation stage perform half a medium change.
8. Cells are ready for continued culture or downstream analysis.

4.5 Shipping Your Cells

Use appropriate controlled room temperature packaging[2] when preparing plates 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 http://pelicanbiothermal.com/thermal-packaging.

5 WellReady™ 96-well plates (WR-N096)

5.1 Components to be Supplied by the User

96-well plate[1] with adherent cell cultures

1000 µL and 200 µL pipettes and tips

Cell culture medium

Multichannel pipette (optional)

Reagent reservoir(s) (optional)

[1]This protocol is intended for use with standard flat bottomed 96 well plates. If your plates are U- or V-bottomed, or will otherwise hold a non-standard volume, please contact technical@atelerix.co.uk for advice on amending the protocol to suit your needs.

5.2 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. Dilute Gel A by adding **3.3mL** of complete culture medium and dilute Gel B and Gel C by adding **8.8mL** of complete culture medium directly to the tube. Mix until homogenous, either on a vortex for 10 seconds or with a pipette 5 – 10 times, ensuring that no bubbles are introduced (see troubleshooting guide on page 10). *N.B. If mixing with a pipette, take extra care to ensure Gel B is homogenous, as it is much more viscous than Gel A and Gel C.*
3. Carefully remove culture medium* from each well of your plate, ensuring that 0.045 mL of culture medium remains in place (*i.e. if the wells contain 0.15 mL medium, remove 0.105 mL*).
4. Add 0.045 mL of the diluted Gel A solution to each well.
5. Gently add 0.09 mL of the diluted Gel B solution on top of the Gel A solution.
6. Add 0.05 mL of Gelation Buffer 1 (**GB1**) dropwise onto the surface of the Gel A/B solutions. Place the lid back onto the plate and allow **10 minutes** for Gelation.

7. Add 0.05 mL of Gelation Buffer 2 (GB2) dropwise onto the surface of the Gel A/B solutions. Place the lid back onto the plate and allow a further 10 minutes for gelation.
8. Avoiding touching the gel, carefully remove GB1/GB2 mixture from each well and wash for 5 minutes with 0.1 mL culture medium per well.
9. Carefully remove the culture medium and add 0.09 mL of the diluted Gel C solution to the centre of each gelled surface.
10. Place the lid back on the plate and store away from light at the recommended temperature for the cell type encapsulated. See the table below 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.

5.3 Cell Storage Temperature Guide

Cell Type	Recommended Storage Conditions	Testing Time of Encapsulation
iPSC-derived Cortical Neurons	15 – 25˚C	5 days

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

5.4 Release

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. Remove the plate seal(s) and, by piercing the surface of the gel with the pipette tip, infuse 0.05 mL of Dissolution Buffer onto the gel within each well of the plate. Allow 10 minutes for gel dissolution.
3. Carefully remove 0.25 mL of the well contents ensuring that you aspirate the liquified gel from the upper part of the well.
4. Add 0.2 mL of complete culture medium and allow to rest 15 minutes for full gel dissolution.
5. Carefully remove 0.2 mL of the well contents.
6. Add 0.2 mL of complete culture medium and return to normal culture conditions for at least 4 hours or overnight.
7. After the incubation stage perform half a medium change.
8. Cells are ready for continued culture or downstream analysis.

5.5 Shipping Your Cells

Use appropriate controlled room temperature packaging[2] when preparing plates 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 http://pelicanbiothermal.com/thermal-packaging.

6 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 layers 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 pipetting.
Do I need to worry about leakage in transit, or keeping the plate the right way up when handling?	No special care is needed when handling the plates once gelation is complete. The gel forms a tight plug which seals the well and is formulated to remain fixed in place, even during the most turbulent transit.
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 plates containing 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 5 and 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.
Can I speed up the WellReady™ process in any way?	Reagent reservoirs and multichannel pipettes can be used to speed up the WellReady™ process. If you have access to an automated liquid handler, please contact us for advice on adapting the protocol at technical@atelerix.co.uk.
Can I split the kit into smaller tubes to get more encapsulations?	We do not recommend removing the gels from their tubes before the addition of media due to their viscosity. Once diluted however you can split the components for use across multiple plates, provided you do not adjust the volumes to attempt to exceed the total number of wells.
If I don't encapsulate a whole plate can I save the diluted gel for use in the future?	The gel volumes supplied are sufficient for a single plate per tube. If you only need to encapsulate part of a plate, the diluted gel is stable for the shelf-life of the diluent used, and within this period is suitable for use for subsequent encapsulations.
Can I use PBS instead of media when washing the gel layers?	No, PBS should not be used at any point as it inhibits and slowly reverses gelation.

7 Statements

7.1 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.

7.2 Cellular Material

This kit can be used to encapsulate adherent cells, cellular monolayers, and 3D cell constructs and models. Please ensure that cell cultures are free of fungal and bacterial contamination before proceeding.

7.3 Trademarks

WellReady™ is a trademark of Atelerix Ltd.

Notes