WELLREADY™

IN-PLATE PRESERVATION OF CARDIOMYOCYTES FOR 48- AND 24-WELL PLATES

WR-C048; WR-C024



Atelerix Handbook Series

Version WR016;0.1.1

TABLE OF CONTENTS

1. Components 1

1.1. Kit Contents 1

1.2. Components to be Supplied by the User 4

1.3. Before You Begin Using WellReady™ 4

2. Protocol overview 4

2.1. Cell Confluence 5

3. WellReady™ 48-Well Plates (WR-C048) 5

3.1. Gelation 5

3.2. Cell Storage Temperature Guide 6

3.3. Release 6

3.4. Shipping your cells 7

4. WellReady™ 24-Well Plates (WR-C024) 7

4.1. Gelation 7

4.2. Cell Storage Temperature Guide 8

4.3. Release 8

4.4. Shipping your cells 9

5. Troubleshooting Guide 10

6. Statements 11

6.1. Kit Storage and Stability 11

6.2. Cellular Material 11

6.3. Trademarks 11

1. COMPONENTS

1.1. KIT CONTENTS

PRODUCT CODE	COMPONENTS	UNITS	UNIT VOLUME	MEDIUM TO ADD
WR-C048-03	Gel A (5x)	3 Tubes	4.4 mL	17.6 mL
	Gel B (5x)	3 Tubes	4.4 mL	17.6 mL
	Gel C (5x)	3 Tubes	4.4 mL	17.6 mL
	Gelation Buffer 1	2 Tubes	16.5 mL	-
	Gelation Buffer 2	2 Tubes	16.5 mL	-
	Dissolution Buffer 1	3 Tubes	20 mL	-
	Dissolution Buffer 2	6 Tubes	20 mL	-
	Adhesive Plate Seals	4	-	-
WR-C048-06	Gel A (5x)	6 Tubes	4.4 mL	17.6 mL
	Gel B (5x)	6 Tubes	4.4 mL	17.6 mL
	Gel C (5x)	6 Tubes	4.4 mL	17.6 mL
	Gelation Buffer 1	3 Tubes	16.5 mL	-
	Gelation Buffer 2	3 Tubes	16.5 mL	-
	Dissolution Buffer 1	6 Tubes	20 mL	-
	Dissolution Buffer 2	12 Tubes	20 mL	-
	Adhesive Plate Seals	7	-	-
WR-C048-12	Gel A (5x)	12 Tubes	4.4 mL	17.6 mL
	Gel B (5x)	12 Tubes	4.4 mL	17.6 mL
	Gel C (5x)	12 Tubes	4.4 mL	17.6 mL
	Gelation Buffer 1	6 Tubes	16.5 mL	-
	Gelation Buffer 2	6 Tubes	16.5 mL	-
	Dissolution Buffer 1	12 Bottles	20 mL	-
	Dissolution Buffer 2	24 Tubes	20 mL	-
	Adhesive Plate Seals	13	-	-
WR-C048-24	Gel A (5x)	24 Tubes	4.4 mL	17.6 mL
	Gel B (5x)	24 Tubes	4.4 mL	17.6 mL
	Gel C (5x)	24 Tubes	4.4 mL	17.6 mL
	Gelation Buffer 1	12 Tubes	16.5 mL	-
	Gelation Buffer 2	12 Tubes	16.5 mL	-
	Dissolution Buffer 1	24 Bottles	20 mL	-
	Dissolution Buffer 2	48 Tubes	20 mL	-
	Adhesive Plate Seals	25	-	-



PRODUCT CODE	COMPONENTS	UNITS	UNIT VOLUME	MEDIUM TO ADD
WR-C048-50	Gel A (5x)	50 Tubes	4.4 mL	17.6 mL
	Gel B (5x)	50 Tubes	4.4 mL	17.6 mL
	Gel C (5x)	50 Tubes	4.4 mL	17.6 mL
	Gelation Buffer 1	24 Tubes	16.5 mL	-
	Gelation Buffer 2	24 Tubes	16.5 mL	-
	Dissolution Buffer 1	50 Tubes	20 mL	-
	Dissolution Buffer 2	100 Tubes	20 mL	-
	Adhesive Plate Seals	52	-	-
WR-C024-03	Gel A (5x)	3 Tubes	4 mL	16 mL
	Gel B (5x)	3 Tubes	4 mL	16 mL
	Gel C (5x)	3 Tubes	4 mL	16 mL
	Gelation Buffer 1	2 Tubes	16.5 mL	-
	Gelation Buffer 2	2 Tubes	16.5 mL	-
	Dissolution Buffer 1	6 tubes	20 mL	-
	Dissolution Buffer 2	6 Tubes	20 mL	-
	Adhesive Plate Seals	4	-	-
WR-C024-06	Gel A (5x)	6 Tubes	4 mL	16 mL
	Gel B (5x)	6 Tubes	4 mL	16 mL
	Gel C (5x)	6 Tubes	4 mL	16 mL
	Gelation Buffer 1	3 Tubes	16.5 mL	-
	Gelation Buffer 2	3 Tubes	16.5 mL	-
	Dissolution Buffer 1	12 Tubes	20 mL	-
	Dissolution Buffer 2	12 Tubes	20 mL	-
	Adhesive Plate Seals	7	-	-
WR-C024-12	Gel A (5x)	12 Tubes	4 mL	16 mL
	Gel B (5x)	12 Tubes	4 mL	16 mL
	Gel C (5x)	12 Tubes	4 mL	16 mL
	Gelation Buffer 1	6 Tubes	16.5 mL	-
	Gelation Buffer 2	6 Tubes	16.5 mL	-
	Dissolution Buffer 1	24 Tubes	20 mL	-
	Dissolution Buffer 2	24 Tubes	20 mL	-
	Adhesive Plate Seals	13	-	-

PRODUCT CODE	COMPONENTS	UNITS	UNIT VOLUME	MEDIUM TO ADD
WR-C024-24	Gel A (5x)	24 Tubes	4 mL	16 mL
	Gel B (5x)	24 Tubes	4 mL	16 mL
	Gel C (5x)	24 Tubes	4 mL	16 mL
	Gelation Buffer 1	12 Tubes	16.5 mL	-
	Gelation Buffer 2	12 Tubes	16.5 mL	-
	Dissolution Buffer 1	48 Tubes	20 mL	-
	Dissolution Buffer 2	48 Tubes	20 mL	-
	Adhesive Plate Seals	25	-	-
WR-C024-50	Gel A (5x)	50 Tubes	4 mL	16 mL
	Gel B (5x)	50 Tubes	4 mL	16 mL
	Gel C (5x)	50 Tubes	4 mL	16 mL
	Gelation Buffer 1	24 Tubes	16.5 mL	-
	Gelation Buffer 2	24 Tubes	16.5 mL	-
	Dissolution Buffer 1	100 Tubes	20 mL	-
	Dissolution Buffer 2	100 Tubes	20 mL	-
	Adhesive Plate Seals	52	-	-

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





1.2. COMPONENTS TO BE SUPPLIED BY THE USER

- 48- or 24-well[1] plate with adherent cell cultures
- 1000µL and 200µL pipettes and tips
- Cell Culture Medium

[1]This protocol is intended for use with standard flat bottomed 48- or 24-well plates. If your plates are U- or V bottomed, or will otherwise hold a non-standard volume, please contact sales@atelerix.co.uk for advice on amending the protocol to suit your needs.

1.3. BEFORE YOU BEGIN USING WELLREADY™

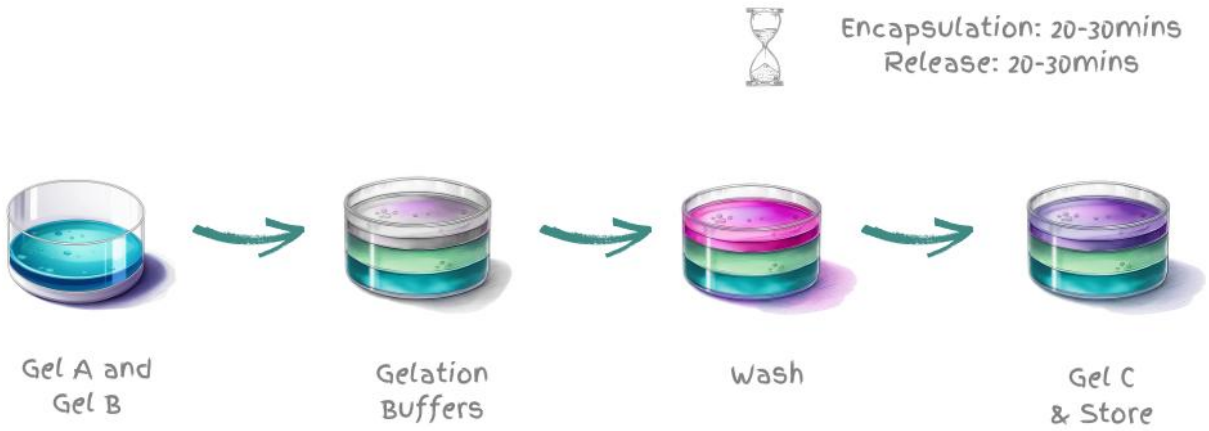
1. Ensure WellReady™ kits have not passed the expiry date stated on the packaging. Atelerix does not recommend using kits after this date.

2. Read the troubleshooting guide on page 10 to see our list of frequently asked questions. For any further queries, please email us at Sales@atelerix.co.uk.

3. Consult the Cell Storage Temperature Guide (page 6 or 8) for the recommended storage conditions per cell type.

4. WellReady™ is intended for use solely in accordance with this protocol using the components provided within the kit.

2. PROTOCOL OVERVIEW



2.1. CELL CONFLUENCE

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

3. WELLREADY™ 48-WELL PLATES (WR-C048)

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. Dilute Gel A, Gel B and Gel C by adding **17.6 mL** of complete culture medium directly to each 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.

4. Add 0.4 mL of the diluted Gel A solution to each well.

5. Gently add 0.4 mL of the diluted Gel B solution on top of the Gel A solution.

6. Add 0.2 mL of Gelation Buffer 1 (**GB1**) dropwise onto the surface of the Gel A/B solutions. Allow 10 minutes for gelation.

7. Add 0.2 mL of Gelation Buffer 2 (**GB2**) dropwise onto the surface of the Gel A/B solutions. 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 0.4 mL culture medium per well.

9. Carefully remove the culture medium and add 0.4 mL 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 in a polystyrene box at the recommended temperature for the cell type encapsulated[1].

11. If shipping your sample, allow plates to rest for **at least 4 hours** before transit for optimal protection. See section 3.4 for shipping recommendations.

[1]See the table on page 6 or, for the most up to date recommendations on storage temperatures and times, visit our Compatibility section on our website or contact sales@atelerix.co.uk.

3.2. CELL STORAGE TEMPERATURE GUIDE

Cell Type	Recommended Storage Conditions	Testing Time of Encapsulation
Hepatocytes	25-35 °C	5 Days
HEK293	15-25 °C	5 Days
Skin Primary Cells	15-25 °C	7 Days

3.3. 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 (20-25°C).

2. Remove the plate seal(s) and, by piercing the surface of the gel with the pipette tip, infuse 0.4 mL of Dissolution Buffer 1 onto the gel within each well of the plate. Allow **10 minutes** for gel dissolution.

3. Carefully remove 0.8 mL of the well contents ensuring that you aspirate the liquified gel from the upper part of the well.

4. Add 0.8 mL of Dissolution Buffer 2 and allow a further **15 minutes** for full gel dissolution.

5. Remove 1.4 mL (remaining contents of each well) and wash monolayers for 5 minutes with 0.4 mL complete culture medium.

6. Add a sufficient volume of complete culture medium and return to normal culture conditions for at least **4 hours** or overnight.

7. Cells are ready for continued culture or downstream analysis.

3.4. SHIPPING YOUR CELLS

Use appropriate controlled 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 ICECATCH Solid Ambient or Cool Shipping Boxes.

Find out more & shop at https://www.atelerix.co.uk/pages/variants-collection-page-accessories

4. WELLREADY™ 24-WELL PLATES (WR-C024)

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. Dilute Gel A, Gel B and Gel C by adding **16 mL** of complete culture medium directly to each 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.

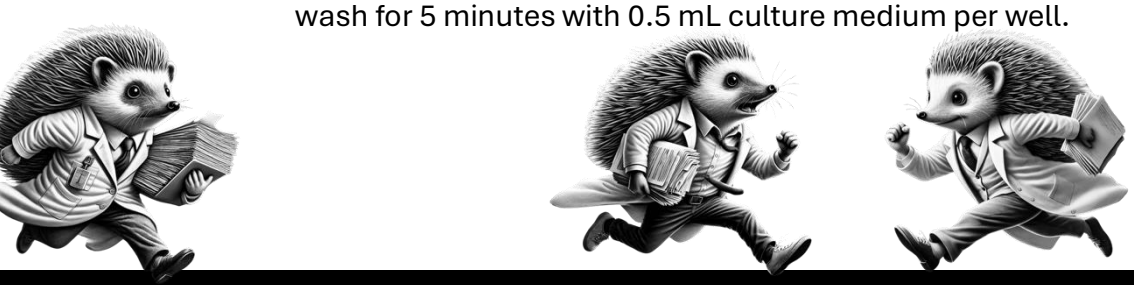
4. Add 0.75 mL of the diluted Gel A solution to each well.

5. Gently add 0.75 mL of the diluted Gel B solution on top of the Gel A solution.

6. Add 0.4 mL of Gelation Buffer 1 (**GB1**) dropwise onto the surface of the Gel A/B solutions. Allow 10 minutes for gelation.

7. Add 0.4 mL of Gelation Buffer 2 (**GB2**) dropwise onto the surface of the Gel A/B solutions. 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 0.5 mL culture medium per well.



9. Carefully remove the culture medium and add 0.75 mL 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 in a polystyrene box at the recommended temperature for the cell type encapsulated[1].

11. If shipping your sample, allow plates to rest for **at least 4 hours** before transit for optimal protection. See section 4.4 for shipping recommendations.

[1]See the table on page 8 or, for the most up to date recommendations on storage temperatures and times, visit our Compatibility section on our website or contact sales@atelerix.co.uk.

4.2. CELL STORAGE TEMPERATURE GUIDE

Cell Type	Recommended Storage Conditions	Testing Time of Encapsulation
Hepatocytes	25-35 °C	5 Days
HEK293	15-25 °C	5 Days
Skin Primary Cells	15-25 °C	7 Days

4.3. 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 (20-25°C).

2. Remove the plate seal(s) and, by piercing the surface of the gel with the pipette tip, infuse 1.5 mL of Dissolution Buffer 1 onto the gel within each well of the plate. Allow **10 minutes** for gel dissolution. *N.B. The volume in the wells will appear full, handle the plate carefully to avoid spillage.*

3. Carefully remove 3 mL of the well contents ensuring that you aspirate the liquified gel from the upper part of the well.

4. Add 1.5 mL of Dissolution Buffer 2 and allow a further **15 minutes** for full gel dissolution.

5. Remove 3 mL (remaining contents of each well) and wash monolayers for 5 minutes with 0.4 mL complete culture medium.

6. Add a sufficient volume of complete culture medium and return to normal culture conditions for at least **4 hours** or overnight.

7. Cells are ready for continued culture or downstream analysis.

4.4. SHIPPING YOUR CELLS

Use appropriate controlled 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 ICECATCH Solid Ambient or Cool Shipping Boxes.

Find out more & shop at https://www.atelerix.co.uk/pages/variants-collection-page-accessories





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.
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 use the kit to encapsulate organoids and / or spheroids?	Yes, you can. We would also recommend TissueReady™ for the encapsulation and storage of organoids and spheroids.
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 our Compatibility section on our website. If you cannot find any recommendations for your cell type, please contact Sales@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 Sales@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 encapsulating samples?	No, PBS should not be used at any point as it inhibits and slowly reverses gelation.

6. STATEMENTS

6.1. KIT STORAGE AND STABILITY

This kit is stable at 2-8°C for up to 10 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

WellReady™ is a trademark of Atelerix Ltd.





NOTES