TISSUEREADY™

PRESERVATION OF TISSUE SAMPLES, BIOPSIES & ORGANOIDS

TR-MNS; TR-LNS; TR-XNS

Atelerix Handbook Series

Version TR07;0.1.1

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1. COMPONENTS

1.1. KIT CONTENTS

PRODUCT CODE	COMPONENTS	UNITS	UNIT VOLUME	MEDIUM TO ADD
TR-MNS-03	GelBase Beads	3 Tubes	0.4 mL	-
	Gel A (5x)	3 Tubes	0.12 mL	0.48 mL
	Dissolution Buffer	3 Tubes	1.1 mL	-
TR-MNS-06	GelBase Beads	6 Tubes	0.4 mL	-
	Gel A (5x)	6 Tubes	0.12 mL	0.48 mL
	Dissolution Buffer	6 Tubes	1.1 mL	-
TR-MNS-12	GelBase Beads	12 Tubes	0.4 mL	-
	Gel A (5x)	12 Tubes	0.12 mL	0.48 mL
	Dissolution Buffer	12 Tubes	1.1 mL	-
TR-MNS-24	GelBase Beads	24 Tubes	0.4 mL	-
	Gel A (5x)	24 Tubes	0.12 mL	0.48 mL
	Dissolution Buffer	24 Tubes	1.1 mL	-
TR-MNS-50	GelBase Beads	50 Tubes	0.4 mL	-
	Gel A (5x)	50 Tubes	0.12 mL	0.48 mL
	Dissolution Buffer	50 Tubes	1.1 mL	-
TR-LNS-03	GelBase Beads	3 Tubes	2 mL	-
	Gel A (5x)	3 Tubes	0.6 mL	2.4 mL
	Dissolution Buffer	3 Tubes	5 mL	-
TR-LNS-06	GelBase Beads	6 Tubes	2 mL	-
	Gel A (5x)	6 Tubes	0.6 mL	2.4 mL
	Dissolution Buffer	6 Tubes	5 mL	-
TR-LNS-12	GelBase Beads	12 Tubes	2 mL	-
	Gel A (5x)	12 Tubes	0.6 mL	2.4 mL
	Dissolution Buffer	12 Tubes	5 mL	-
TR-LNS-24	GelBase Beads	24 Tubes	2 mL	-
	Gel A (5x)	24 Tubes	0.6 mL	2.4 mL
	Dissolution Buffer	24 Tubes	5 mL	-
TR-LNS-50	GelBase Beads	50 Tubes	2 mL	-
	Gel A (5x)	50 Tubes	0.6 mL	2.4 mL
	Dissolution Buffer	50 Tubes	5 mL	-

PRODUCT CODE	COMPONENTS	UNITS	UNIT VOLUME	MEDIUM TO ADD
TR-XNS-03	GelBase Beads	3 Tubes	0.4 mL	-
	Gel A (5x)	3 Tubes	0.12 mL	0.48 mL
	Dissolution Buffer	3 Tubes	1.1 mL	-
TR-XNS-06	GelBase Beads	6 Tubes	0.4 mL	-
	Gel A (5x)	6 Tubes	0.12 mL	0.48 mL
	Dissolution Buffer	6 Tubes	1.1 mL	-
TR-XNS-12	GelBase Beads	12 Tubes	0.4 mL	-
	Gel A (5x)	12 Tubes	0.12 mL	0.48 mL
	Dissolution Buffer	12 Tubes	1.1 mL	-
TR-XNS-24	GelBase Beads	24 Tubes	0.4 mL	-
	Gel A (5x)	24 Tubes	0.12 mL	0.48 mL
	Dissolution Buffer	24 Tubes	1.1 mL	-
TR-XNS-50	GelBase 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 for at least 20 minutes before use.

1.2. COMPONENTS TO BE SUPPLIED BY THE USER

• 1000µl Pipettes and Tips • Cell Culture Medium (Antibiotic-Antimycotic supplementation is recommended if used outside of a sterile environment) • Sterile forceps or Pasteur pipette • Syringe and needle (optional) • Tissue samples, biopsies or organoids!!

1.3. BEFORE YOU BEGIN USING TISSUEREADY™

1. Ensure TissueReady™ 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 14 to see our list of frequently asked questions. For any further queries, please email us at Sales@atelerix.co.uk.

3. Consult the Sample Size Guide for Fresh Tissues and Organoids & Spheroids on page 3-4.

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

2. SAMPLE SIZE GUIDE

2.1. FRESH TISSUES

Product	Range	Tissue Size (Length x Width x Depth)
TissueReady™-M (TR-MNS)	Lower Limit	None
	Upper Limit	≤ 0.5cm x 0.5cm x 0.5cm
TissueReady™-L (TR-LNS)	Lower Limit	> 0.5cm x 0.5cm x 0.5cm
	Upper Limit	≤ 3.0cm x 0.9cm x 0.9cm
TissueReady™-XL (TR-XNS)	Lower Limit	> 3.0cm x 0.9cm x 0.9cm
	Upper Limit	≤ 4.0cm x 1.6cm x 1.6cm

2.2. ORGANOIDS AND SPHEROIDS

Product	Organoid/Spheroid Size	Recommended Number/Vial
TissueReady™-M (TR-MNS)	> 150µm ≤ 300µm	≤ 0.5×10^6
TissueReady™-L (TR-LNS)	> 150µm ≤ 300µm	≤ 2.5×10^6
TissueReady™-XL (TR-XNS)	> 150µm ≤ 300µm	≤ 11.25×10^6

3. PROTOCOL OVERVIEW

4. TISSUEREADY™ MEDIUM (TR-MNS)

4.1. GELATION

Caution: Follow the correct gelation step depending on the sample being stored - Fresh Tissue (4.1.1) OR Organoids & Spheroids (4.1.2).

4.1.1. GELATION FOR FRESH TISSUES

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. Add 0.48 mL cell culture medium to the vial containing 0.12 mL of Gel A.

3. Gently mix until homogenous, with a pipette, ensuring that no bubbles are introduced (see troubleshooting guide on page 14).

4. Add 0.6 mL of the medium / Gel A mix to the GelBase Beads.

5. 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 contents and proceed immediately to **step 6**.

6. Remove the cap and add your fresh tissue sample into the **gel / bead** mixture, ensuring that it is entirely submerged. [1]This must be done within **20 minutes** of step 5.

7. Place the cap back on the tube, ensuring a tight seal (the gel will cure in situ within approximately 30 minutes, sample is ready to ship after 1 hour).

8. Store away from light in a polystyrene box at the recommended temperature for the tissue type encapsulated. See the table on page 7 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.

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

4.1.2. GELATION FOR ORGANOIDS & SPHEROIDS

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 organoids in 0.48 mL cell culture medium.

3. Add 0.48 mL of organoid suspension directly to the vial containing 0.12 mL Gel A. Gently mix until homogenous, using a Pasteur or micropipette 5 – 10 times, ensuring that no bubbles are introduced (see troubleshooting guide on page 14).

4. Add 0.6 mL of the organoid suspension / Gel A mix to the GelBase Beads.

5. 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 contents (the gel will cure in situ within approximately 30 minutes, sample is ready to ship after 1 hour).

6. Store away from light in a polystyrene box at the recommended temperature for the tissue type encapsulated. See the table on page 7 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.

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

4.2. STORAGE TEMPERATURE GUIDE

Sample	Output	Recommended Storage Conditions	Tested Storage Time
Tissue	Histology	2 - 8°C	2-3 Days
Tissue	Cell Viability	15 – 25°C	4-5 Days
Tissue	Histology & Cell Viability	15 – 25°C	2-3 Days
Organoids	Cell Viability	15– 25°C	6 Days

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 serological pipette or syringe with needle, pierce the gel and infuse 1 mL Dissolution Buffer into the bottom of the gel by piercing the gel, filling up to the indicated line. As the Dissolution Buffer is added to the gel, remove the pipette tip/needle to avoid spillage and ensure that you do not disturb the sample.

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 14).

4. When the gel has fully dissolved, the sample can be recovered using sterile forceps (if preserving organoids, collect using a Pasteur or micropipette).

5. Wash sample with culture medium or buffer before continuing with downstream processes.

4.4. SHIPPING YOUR SAMPLE

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.

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5. TISSUEREADY™ LARGE (TR-LNS)

5.1. GELATION

Caution: Follow the correct gelation step depending on the sample being stored - Fresh Tissue (5.1.1) OR Organoids & Spheroids (5.1.2).

5.1.1. GELATION FOR FRESH TISSUES

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. Add 2 mL cell culture medium to the vial containing 0.5 mL of Gel A.

3. Gently mix until homogenous, with a pipette, ensuring that no bubbles are introduced (see troubleshooting guide on page 14).

4. Add 2.5 mL of the medium / Gel A mix to the GelBase Beads.

5. 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 contents and proceed immediately to **step 6**.

6. Remove the cap and add your fresh tissue sample into the **gel / bead** mixture, ensuring that it is entirely submerged. [1]This must be done within **20 minutes** of step 5.

7. Place the cap back on the tube, ensuring a tight seal (the gel will cure in situ within approximately 30 minutes, sample is ready to ship after 1 hour).

8. Store away from light in a polystyrene box at the recommended temperature for the tissue type encapsulated. See the table on page 9 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.

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

5.1.2. GELATION FOR ORGANOIDS & SPHEROIDS

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 organoids in 2 mL cell culture medium.

3. Add 2 mL of organoid suspension directly to the vial containing 0.5 mL Gel A. Gently mix until homogenous, using a Pasteur or micropipette 5 – 10 times, ensuring that no bubbles are introduced (see troubleshooting guide on page 14).

4. Add 2.5 mL of the organoid suspension / Gel A mix to the GelBase Beads.

5. 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 contents (the gel will cure in situ within approximately 30 minutes, sample is ready to ship after 1 hour).

6. Store away from light in a polystyrene box at the recommended temperature for the tissue type encapsulated. See the table on page 9 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.

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

5.2. STORAGE TEMPERATURE GUIDE

Sample	Output	Recommended Storage Conditions	Tested Storage Time
Tissue	Histology	2 - 8°C	2-3 Days
Tissue	Cell Viability	15 – 25°C	4-5 Days
Tissue	Histology & Cell Viability	15 – 25°C	2-3 Days
Organoids	Cell Viability	15– 25°C	6 Days

5.3. 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 serological pipette or syringe with needle, pierce the gel and infuse 3 mL Dissolution Buffer into the bottom of the gel by piercing the gel, filling up to the indicated line. As the Dissolution Buffer is added to the gel, remove the pipette tip/needle to avoid spillage and ensure that you do not disturb the sample.

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 14).

4. When the gel has fully dissolved, the sample can be recovered using sterile forceps (if preserving organoids, collect using a Pasteur or micropipette).

5. Wash sample with culture medium or buffer before continuing with downstream processes.

5.4. SHIPPING YOUR SAMPLE

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.

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6. TISSUEREADY™ (TR-XNS)

6.1. GELATION

Caution: Follow the correct gelation step depending on the sample being stored - Fresh Tissue (6.1.1) OR Organoids & Spheroids (6.1.2).

6.1.1. GELATION FOR FRESH TISSUES

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. Add 8 mL cell culture medium to the vial containing 2 mL of Gel A.

3. Gently mix until homogenous, with a pipette, ensuring that no bubbles are introduced (see troubleshooting guide on page 14).

4. Add 10 mL of the medium / Gel A mix to the GelBase Beads.

5. 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 contents and proceed immediately to **step 6**.

6. Remove the cap and add your fresh tissue sample into the **gel / bead** mixture, ensuring that it is entirely submerged. [1]This must be done within **20 minutes** of step 5.

7. Place the cap back on the tube, ensuring a tight seal (the gel will cure in situ within approximately 30 minutes, sample is ready to ship after 1 hour).

8. Store away from light in a polystyrene box at the recommended temperature for the tissue type encapsulated. See the table on page 12 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.

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



6.1.2. GELATION FOR ORGANOIDS & SPHEROIDS

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 organoids in 8 mL cell culture medium.

3. Add 8 mL of organoid suspension directly to the vial containing 2 mL Gel A. Gently mix until homogenous, using a Pasteur or micropipette 5 – 10 times, ensuring that no bubbles are introduced (see troubleshooting guide on page 14).

4. Add 10 mL of the organoid suspension / Gel A mix to the GelBase Beads.

5. 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 contents (the gel will cure in situ within approximately 30 minutes, sample is ready to ship after 1 hour).

6. Store away from light in a polystyrene box at the recommended temperature for the tissue type encapsulated. See the table on page 12 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.

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

6.2. STORAGE TEMPERATURE GUIDE

Sample	Output	Recommended Storage Conditions	Tested Storage Time
Tissue	Histology	2 - 8°C	2-3 Days
Tissue	Cell Viability	15 – 25°C	4-5 Days
Tissue	Histology & Cell Viability	15 – 25°C	2-3 Days
Organoids	Cell Viability	15– 25°C	6 Days

6.3. 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 serological pipette or syringe with needle, pierce the gel and infuse 3 mL Dissolution Buffer into the bottom of the gel by piercing the gel, filling up to the indicated line. As the Dissolution Buffer is added to the gel, remove the pipette tip/needle to avoid spillage and ensure that you do not disturb the sample.

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 14).

4. When the gel has fully dissolved, the sample can be recovered using sterile forceps (if preserving organoids, collect using a Pasteur or micropipette).

5. Wash sample with culture medium or buffer before continuing with downstream processes.

6.4. SHIPPING YOUR SAMPLE

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.

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7. 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?	We would 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 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 GelBase Beads from the tubes supplied or deviating from the volumes stated.
Can I encapsulate multiple tissue biopsies in the same tube in order to get more encapsulations?	Yes, multiple tissues may be encapsulated in the same tube provided the combined size of the tissues does not exceed the recommended tissue size, and all pieces of tissue can be fully submerged in the gel.
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 Sales@atelerix.co.uk for information on how long cells may be allowed to incubate in the Dissolution Buffer.

8. STATEMENTS

8.1. KIT STORAGE AND STABILITY

This kit is stable at 2-8°C for up to 12 months. Atelerix does not recommend using the kit after the expiry date stated on the packaging.

8.2. CELLULAR MATERIAL

Please ensure that biological materials are free of fungal and bacterial contamination before proceeding.

8.3. TRADEMARKS

TissueReady™ is a trademark of Atelerix Ltd.

If you cannot find any recommendations for your tissue, organoid, or spheroid type, please visit our Compatibility section on our website or contact Sales@atelerix.co.uk.

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