

TR-MNS

Preservation of Small Tissue Samples and Biopsies

# Table of Contents

1	C	omponents	1
	1.1	Kit Contents	1
	1.2	Components to be Supplied by the User	2
	1.3	Before You Begin using TissueReady™	2
2	St	ep-by-Step Guide	3
	2.1	Overview	3
	2.2	Sample Size Guide	3
	2.3	Gelation	4
	2.4	Storage Temperature Guide	5
	2.5	Release	5
	2.6	Shipping Your Samples	5
	2.7	Troubleshooting Guide	6
3	St	atements	7
	3.1	Kit Storage and Stability	7
	3.2	Cellular Material	7
	3.3	Trademarks	7

# 1 Components

### 1.1 Kit Contents

Product Code	Components	Units	Unit Volume	Medium to Add
	GelBase Beads	3 tubes	0.4 mL	-
TR-MNS-03	Gel A (5x)	3 tubes	0.12 mL	0.48 mL
	Dissolution Buffer	3 tubes	1.1 mL	-
	GelBase Beads	6 tubes	0.4 mL	-
TR-MNS-06	Gel A (5x)	6 tubes	0.12 mL	0.48 mL
	Dissolution Buffer	6 tubes	1.1 mL	-
	GelBase Beads	12 tubes	0.4 mL	-
TR-MNS-12	Gel A (5x)	12 tubes	0.12 mL	0.48 mL
	Dissolution Buffer	12 tubes	1.1 mL	-
	GelBase Beads	24 tubes	0.4 mL	-
TR-MNS-24	Gel A (5x)	24 tubes	0.12 mL	0.48 mL
	Dissolution Buffer	24 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
Sterile forceps or Pasteur pipette

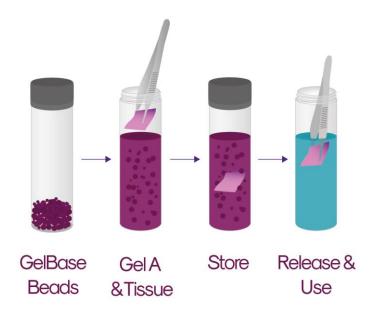
#### 1.3 Before You Begin using TissueReady™

- 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 <a href="https://www.atelerix.co.uk/technical-support-questionnaire/">https://www.atelerix.co.uk/technical-support-questionnaire/</a>.
- 2. Ensure TissueReady™ kits have not passed the expiry date stated on the packaging.

  Atelerix does not recommend using kits after this date.
- 3. Visit <a href="https://www.youtube.com/watch?v=3WgXWOmDK9U">https://www.youtube.com/watch?v=3WgXWOmDK9U</a> and watch our TissueReady™ video protocol.
- 4. Read the troubleshooting guide on page 6 to see our list of frequently asked questions. For any further queries, please email us at <a href="technical@atelerix.co.uk">technical@atelerix.co.uk</a>.
- 5. TissueReady™ 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 Sample Size Guide

Range	Tissue Size (length x width x depth)	Tissue volume allowance
Lower Limit	none	none
Upper Limit	≤ 0.5cm x 0.5cm x 0.5cm	≥ 0.001cm <sup>3</sup>

#### 2.3 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 1 in 5 with cell culture medium by adding directly to the tube containing the gel concentrate (See kit contents on Page 1 for a dilution guide). 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 6).
- 3. Add 0.6 mL of diluted Gel A to the tube of GelBase Beads. Place the cap back on the tube and invert the gel / bead mixture several times until the beads are evenly distributed throughout the gel. Gently flick the tube to settle the contents and proceed *immediately* to step 4.
- 4. Remove the cap and add your sample into the **gel / bead** mixture, ensuring that it is entirely submerged.<sup>1</sup> This must be done within **20 minutes** of step 3.
- 5. 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).
- 6. Store away from light at the recommended temperature for the tissue 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-testingconditions/ or by scanning the QR code.

<sup>1</sup>Use the collection tube containing Gelbase Beads provided for encapsulation, storage, and release.



#### 2.4 Storage Temperature Guide

Sample	Recommended Storage Conditions	Testing Time of Encapsulation
Tissue	15 - 25°C	7 days
Organoids / Spheroids	20°C	5 days

#### 2.5 Release

- 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 a needle with a syringe, infuse 1 mL Dissolution Buffer into the gel, ensuring 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 6). When the gel has fully dissolved the sample can be recovered using sterile forceps or a Pasteur pipette.
- 4. Wash sample with culture medium or buffer solution before continuing with downstream processes.

### 2.6 Shipping Your Samples

Use appropriate controlled room temperature packaging<sup>2</sup> when preparing samples for shipping to reduce the effect of ambient temperature change on the encapsulated tissues or organoids / spheroids during transit.

<sup>2</sup>For best cell recovery upon arrival, we recommend using the CoolGuard™ Advance CRT container. Find out more at <a href="http://pelicanbiothermal.com/thermal-packaging">http://pelicanbiothermal.com/thermal-packaging</a>.

# 2.7 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?	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 tissue type?	A guide to the recommended storage times and temperatures can be found on page 4 of this book and at <a href="https://www.atelerix.co.uk/guidelines-for-testing-conditions/">https://www.atelerix.co.uk/guidelines-for-testing-conditions/</a> If you cannot find any recommendations for your cell type please contact <a href="mailto:technical@atelerix.co.uk">technical@atelerix.co.uk</a> .
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 beads have not fully dissolved after 10 minutes? Can the sample be allowed to sit in the buffer for longer?	As long as the Gel A component is dissolved you do not need to wait for the beads to dissolve to remove your sample (unless your sample is organoids / spheroids). Please contact technical@atelerix.co.uk for information on how long materials 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

This kit can be used to encapsulate small tissue biopsies and cellular spheroids/organoids.<sup>2</sup> Please ensure that biological materials are free of fungal and bacterial contamination before proceeding.

#### 3.3 Trademarks

TissueReady™ is a trademark of Atelerix Ltd.

### Notes