Jellagen

MARINE BIOTECHNOLOGIES

JellaGel™ Hydrogel Kit: Gelling Protocol

- Please consult the 'User Guide' for volume ratios to use JellaGel in various formats.
- Prior to use, remove a frozen aliquot of Crosslinker solution from the freezer and thaw at room temperature.
- For a single-use experiment, use cold water to prepare the Crosslinker solution immediately prior to use.
- For pH verification pH paper can be used.
- Prepare your cells during the 1 hour wait period, ready for the addition of the JellaGel solution. As a guide you can add between 100,000 and 500,000 cells for every mL of JellaGel, however, this will require optimization according to your application.

ALL STEPS TO BE CARRIED OUT AT ROOM TEMPERATURE

Step 1

1.1 Add JellaGel Solution to an appropriate mixing vessel that will allow easy solution mixing.

1.2 Then add 120 µL of buffer solution for every 1 mL of JellaGel solution and mix thoroughly.

Optional: pH Check

Following addition of the correct buffer volume (120uL buffer to each 1mL of JellaGel), the solution will be at neutral pH containing isotonic salt levels.

If desired the pH can be verified by aliquoting a small volume to wet a pH test strip.

Always avoid introducing bubbles whenever mixing JellaGel–try swirling and inverting. **Do not vortex!**

Optional: Dilution
Dilution of the buffered Jellagen solution can be conducted using sterile phosphate buffered saline (PBS). Up to 0.5 mL PBS can be added for every 1 mL of JellaGel solution.

Step 2

Wait for 1 hour between steps 1 and 3 using this time to prepare your cells.

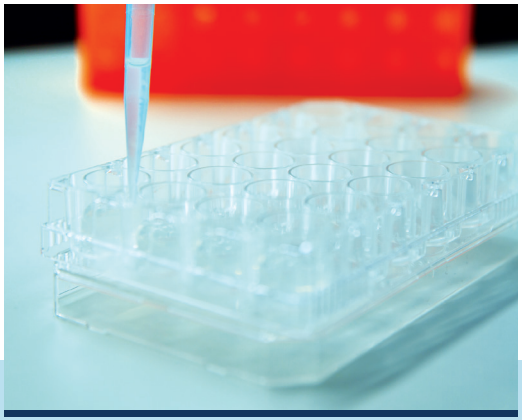
2.1 Remove excess medium from centrifuged cell pellet.

2.2 Add neutralized JellaGel solution to the cell pellet and gently resuspend cells.

Step 3

Add 100 µL of crosslinker solution (prepared according to the 'User Guide') for each 1mL of JellaGel solution and mix thoroughly.

Step 4



Transfer solution into TC-coated well plate, transwell insert or imaging plate of choice.

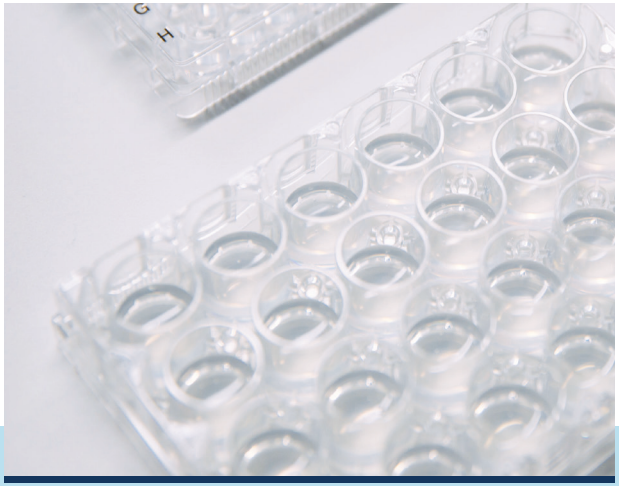
Step 5

Leave at room temperature for 15 min and then incubate at 37 °C for 30 min (in a 5% CO_2 incubator).

Step 6

After hydrogel formation, gently cover with cell culture medium of choice (or add into the wells for a transwell experiment).

This can be repeated periodically according to standard cell culture practices to maintain pH and fresh nutrients.



Gels should be translucent and have minimal bubbles.

JellaGel User Guide

Product Code: JGEL10ML

JellaGel Kit contains:

- **10 mL JellaGel solution**
- **Buffer solution**
- **Crosslinker**

Following this protocol yields a maximum total working volume of just over 12 mL of hydrogel.

To assist in your experimental design, we have projected how many individual gels (of 2mm thickness/height) can be generated using a single hydrogel kit:

Format	Internal diameter of well / cm	Culture area per well / cm2	Volume of hydrogel* / µL	Max number of hydrogels**
96 well plate	0.68	0.4	73	160
96 transwell insert	0.43	0.1	29	400
24 well plate	1.62	2.1	412	28
24 transwell insert	0.65	0.3	66	180
6 well plate	3.46	9.4	1880	6
6 transwell insert	2.40	4.5	904	12
8 well imaging plate	-	1.0	200	55

**Hydrogel volume (in µL) calculated using: Area per well (in cm^2) x 0.2 cm height x 1000*

*** Maximum number of hydrogels accounts for small losses of volume during pipetting – this will vary depending on the scale of experiments being performed.*

This is provided as a guide, if you want to make hydrogels in a different format or of a different thickness then these figures will need to be adjusted accordingly. Your experimental design will need to be tailored to the requirements of your analytical techniques and/or cells of interest.

Having decided upon an experimental design, you can then determine how many individual experiments you will conduct with a single hydrogel kit. Follow the instructions below for single or multiple uses.

IMPORTANT: The Crosslinker, once dissolved in water, needs to be used immediately or frozen into single-use aliquots. Frozen aliquots of Crosslinker solution must be used within '2 weeks'. It is important that you consider this in advance of conducting your lab work. To assist you in this, please follow the guidelines:

1. For single use

1.1 Remove the Crosslinker vial from the freezer, add 1.2 mL of refrigerated cell culture grade water and vortex to dissolve.

1.2 Use immediately for the formation of hydrogels according to the protocol.

2. For multiple uses

2.1 Determine how many individual experiments you will perform with a single kit.

2.2 Follow step 1.1 and then immediately aliquot the crosslinker solution into sterile microcentrifuge tubes (one for each experiment) and freeze for future use.

2.3 Immediately prior to each experiment thaw a crosslinker aliquot and use directly. Do not leave thawed aliquots for later use.

Example

To conduct three separate experiments (each comprising 9 gels in a 24 well format) dissolve the crosslinker to 1.2mL and aliquot into 3 x 400 µL aliquots and freeze for later use. According to the table above and the JellaGel protocol, use 3.33 mL of JellaGel solution and combine with 400 µL of buffer. Suspend your cells and introduce 333 µL of freshly thawed crosslinker solution. Transfer 412 µL of JellaGel into 9 separate wells (totaling 3.7 mL) and leave at room temperature for 15 mins before placing in a tissue culture incubator for hydrogel formation. After 30 minutes fresh medium can be carefully placed on top of the hydrogels to ensure constant pH and nutrient supply to cells.