Jellagen

NEXT GENERATION COLLAGEN

JellaGel™ Hydrogel Kit: Gelling Protocol

- Please consult the 'User Guide' on the back of this protocol for crosslinker preparation.
- Immediately prior to use, remove a frozen aliquot of crosslinker solution from the freezer and thaw at room temperature.
- Higher concentration JellaGel offers the user the ability to tune physical properties (e.g. stiffness and density) by varying the concentration of components (e.g. crosslinker and/or collagen solution).
- This is a suggested protocol and all parameters can be adapted at the users discretion.
- This protocol is suitable for all standard (JGEL) and high concentration (JGELC) JellaGel products.

ALL STEPS TO BE CARRIED OUT AT ROOM TEMPERATURE

Protocol

Step 1

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

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

Optional: pH Check

Following addition of the correct buffer volume (120µL buffer to each 1mL of JellaGel), the solution should be pH 7.3-7.6.

The pH can be verified and adjusted as necessary (for example, using concentrated NaOH)

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

Optional: Dilution
Dilution of the buffered JellaGel solution can be conducted by the user. The composition and amount of dilution should be determined by the user.

Step 2

2.1 Wait 60 minutes. Use this time to prepare your cells.

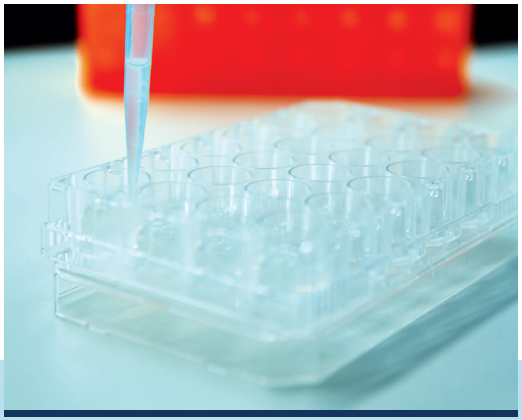
2.2 Add 100µL of crosslinker solution (prepared according to the 'User Guide') for each 1mL of JellaGel solution and mix thoroughly.

Step 3

3.1 Wait for 2-3 minutes, during which time remove excess medium from cells.

3.2 Use JellaGel solution to gently resuspend the cell pellet.

Step 4



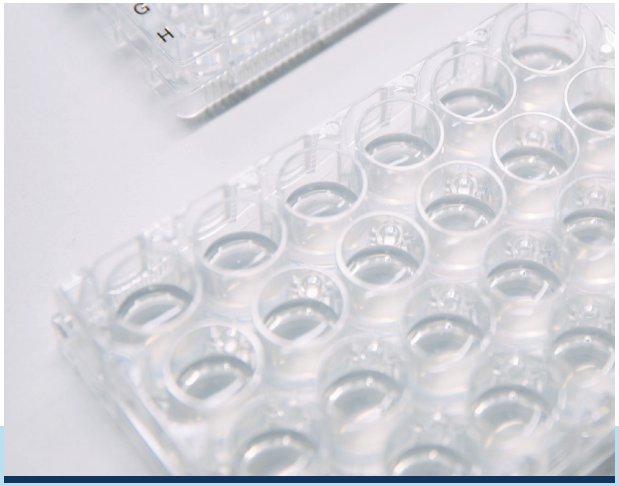
4.1 Immediately transfer JellaGel cell suspension into TC-coated well plate, transwell insert or imaging plate of choice.

Step 5

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

Step 6

6.1 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

JellaGel Kit contains:

- JellaGel solution
- Buffer solution
- Crosslinker

Using the mixing ratios outlined in the protocol, the user should decide the total volume of JellaGel that is suitable for their experiment based on the number of gels to be formed and the volume desired per gel.

Tuning JellaGel is best suited to the high concentration version (JGELC).

Crosslinker Preparation

IMPORTANT: The crosslinker, once dissolved in water, needs to be used immediately or aliquoted and frozen immediately.

Multiple aliquots of crosslinker may be supplied in each kit. The user should store these in the freezer until ready to use. They can be dissolved one at a time, when needed. Frozen aliquots of crosslinker solution must be used within 2 weeks (longer periods may be used at the user's risk). It is important that you consider this in advance of conducting your lab work. To assist you in this, please follow the guidelines:

Standard Preparation

1. Remove the crosslinker vial from the freezer, add 1.2mL of refrigerated cell culture grade water and vortex to dissolve.
2. Use immediately for the formation of hydrogels according to the protocol.

Tuning Preparation

A strategy for varying the physical properties of JellaGel is to control the amount of crosslinker. The following steps can be taken to either increase or decrease the amount of crosslinker. This is provided as a guide and must be developed by the user for their specific application and needs.

1 The user can dissolve the frozen aliquot of crosslinker by adding 1.2mL of refrigerated cell culture grade water and vortexing to dissolve. This is referred to as **1X concentration.**

2. To increase the stiffness of the gel the user can (for example) reduce the amount of water added to the crosslinker to 0.6mL - achieving a **2X concentration** hydrogel.
3. To decrease the stiffness of the hydrogel the user may reduce the volume of crosslinker added in step 2.2 to, (for example), 50 uL per 1mL of Jellagel solution. This would achieve a hydrogel with **0.5X concentration.**
4. Whenever preparing the crosslinker solution, use it immediately or aliquot and freeze immediately.

As indicated in Step 1 of the protocol, the user may also dilute JellaGel in order to reduce the overall stiffness of the matrix. Certain buffers or growth media will not be compatible with JellaGel (i.e. those that contain amino groups or other proteins), please contact us for guidance.

For technical support, please contact us.

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