PeptiMatrix™
Next generation peptide hydrogels

STANDARD OPERATING PROCEDURE

ENCAPSULATING CELLS IN PEPTIMATRIX™ HYDROGELS

1. BACKGROUND

Self-assembling peptide hydrogels can be used in a variety of cell culture applications, providing a 3D environment in which cells can be encapsulated. PeptiMatrix™ hydrogels are formed from short peptide sequences of between 8-20 amino acids. At the correct pH and temperature, they self-assemble to form a stable entangled network of nanofibers. The non-covalent nature of the peptide interactions enables the hydrogel to undergo gel-liquid-gel transitions upon shear stress, allowing for uniform cell encapsulation.

2. RISK ASSESSMENT

Always follow your organisation's laboratory safety procedures.

Work inside an appropriate microbiology safety cabinet for your cell type. Refer to the **PeptiMatrix Safety Data Sheet (SDS)** for detailed safety, handling, storage, and first aid information relating to the hydrogel components.

If you are working with additional cell lines, media supplements, matrix additives, or other reagents, consult the relevant SDS documents for those materials as well.

3. MATERIALS

- PeptiMatrix hydrogel
- Class II microbiology safety cabinet (or appropriate class for your cell type)
- Centrifuge and 15 mL centrifuge tubes
- 80 °C oven (optional, for older gels)
- P1000, P200 pipettes and filter tips
- 96-well plate
- Cells for encapsulation
- Soluble matrix additions (optional)

4. METHODS

4.1 Preparing the hydrogel

This procedure can be scaled up or down depending on the number of wells you plan to prepare, and which well plate size you are using.

The key requirement is to maintain a **4:1 ratio of hydrogel to cell suspension** to ensure correct gelation.

For reference, the steps below describe the process using **1 mL of hydrogel** plated into a **96-well plate**.

1. Transfer 1 mL of PeptiMatrix hydrogel into a 15 mL centrifuge tube.
2. Centrifuge at 600 x g for 1 minute to remove any trapped air bubbles.

Optional: Restoring older gels

If the hydrogel has been stored for longer than one month:

1. Place the tube in an 80 °C oven for at least 20 minutes to improve homogeneity.
    - Gels of lower concentration will become free flowing at this temperature.
    - Higher concentration gels may remain self-supporting but will be less viscous.
    - Do not exceed 90 °C. Higher temperatures can damage the gel.
2. Allow to cool, then vortex for 1 minute.
3. Centrifuge at 600 x *g* for 1 minute to remove any trapped air bubbles.

If the hydrogel appears slightly cloudy after storage, this can usually be corrected by adjusting the pH:

1. Place the tube in at 80 °C oven for at least 20 minutes to improve homogeneity.
2. Allow to cool, then for every 1 mL of hydrogel, add 1 µL of 0.5M NaOH.
3. Vortex for 1 minute, then centrifuge to remove bubbles.
4. Visually inspect the gel. Repeat steps 1-3 as required to until gel becomes fully transparent again (typical recovery is 1-3 cycles).
5. It is important not to overcorrect, as this will cause the gel to undergo gel-liquid transition and cannot be recovered after this point.

Cloudiness is typically caused by a gradual pH drift during storage or exposure to air. Adding a small amount of 0.5 M NaOH restores the pH balance and returns the gel to its transparent state.

4.2 Preparing the cell suspension

1. Detach and count cells using your standard protocol.
2. Prepare **250 µL** of cell suspension per gel at your chosen concentration.
    - A good starting point is **2.5 x 10^5 cells per mL of hydrogel**. This requires preparing the cell suspension at 1 x 10^6 cells per mL, as this will be effectively diluted when mixing with the hydrogel.
    - Depending on your cell type, **1 x 10^4 to 1 x 10^6 cells per mL of hydrogel** may be more suitable.
    - To easily work out how to prepare you stock cell suspension to reach a desired concentration in our hydrogels, you may find our **Cell concentration calculator** useful: https://www.peptimatrix.com/cell-calculator

Optional: Adding soluble matrix components

If you wish to introduce soluble ECM proteins or other additives:

1. Resuspend the cell pellet in a mixture of the matrix component and media, totalling 250 µL.
2. Keep the suspension on ice if the additives gel at room temperature.

Suggestions for suitable ECM proteins and other matrix additions are provided in **Section 7**.

4.3 Mixing cells with the hydrogel

There are two methods commonly used for mixing cells with the hydrogel. For higher concentration and more viscous hydrogels we recommend **Option B**.

Option A

1. Using a P1000 pipette, add the cell suspension dropwise onto the surface of the hydrogel.
2. Change the pipette tip.
3. Mix the cells and gel gently by slow pipetting and stirring.
    - Reverse pipetting (set to 200 µL) helps avoid air bubbles.
    - For a description of reverse pipetting see **Section 6** below.
4. Once fully mixed, proceed to plating.

A demonstration video of the above procedure is available on YouTube: *How to create 3D cell culture models with PeptiMatrix hydrogels*

Option B

1. Using a P1000 pipette, slowly dispense 250 µL of cell suspension into the gel, starting near the bottom of the tube and gradually drawing the pipette tip upward. Mix gently in a corkscrew motion as you dispense.
2. Change the pipette tip.
3. Mix the cells and gel gently by slow pipetting and stirring.
    - Reverse pipetting (set to 200 µL) helps avoid air bubbles.
4. Once fully mixed, proceed to plating.

4.4 Plating the gels

1. Using a P200 pipette, dispense the cell-gel mixture into a 96-well plate. Add at least 50 µL to the centre of each well. Complete coverage is important for consistent gelation. If this is your first time, adding 100 µL is generally easier to ensure complete coverage.
2. Incubate the gels at 37 °C for 10 minutes to complete gelation.

This procedure can be scaled to other plate formats, provided that sufficient gel is added to completely cover the base of the well or insert. Suggested volumes are given below:

Plate size	Approx. volume per well
384-well plate	10 – 35 µL
96-well plate	50 – 100 µL
48-well plate	150 – 250 µL
24-well plate insert	50 – 100 µL
24-well plate	0.3 – 0.5 mL
12-well plate	0.5 mL
6-well plate	1 – 2 mL

4.5 Adding media

1. Add up to 200 µL of media to each well of a 96-well plate dropwise on top of each plated gel.
2. Change the media the next day, then according to the needs of your cell type. When changing media, exchange only 75% of the total volume to minimise risk of disturbing the gel.

For other well plates, adjust volume of media added according to the remaining working volume for each well.

Optional: Working with cells sensitive to pH

PeptiMatrix hydrogels are slightly alkaline before mixing with media. During encapsulation, the gels neutralise rapidly to around pH 9 and then gradually reach pH 7 over approximately 12 hours.

If your cells are particularly sensitive to pH changes, performing two media changes within the first hour after plating can help the gels reach physiological pH more quickly.

This step is generally unnecessary for robust cancer cell lines but can be helpful for sensitive primary cells and stem cell lines.

5. DISPOSAL

Dispose of hydrogels containing cells, media, or matrix components according to your local guidelines for biological waste.

6. HOW TO REVERSE PIPETTE

Reverse pipetting is recommended for viscous liquids such as hydrogels. It aspirates a slightly larger volume than needed, which improves precision when dispensing and helps to avoid air bubbles.

How to reverse pipette:

1. Set the desired volume on the pipette.
2. Attach a suitable tip.
3. Press the plunger to the **second stop**.
4. Submerge the tip just below the surface of the liquid.
5. Slowly release the plunger to the **starting position**.
    - Wait a few seconds if the liquid is very viscous.
6. Withdraw the tip.
7. Dispense to the **first stop** to deliver the desired volume.
8. Remove the tip and press to the **second stop** to discard the excess liquid.

A demonstration video is available on YouTube: *Tutorial: How to Reverse Pipette?*

7. MATRIX ADDITIONS

The PeptiMatrix system is compatible with a wide range of extracellular matrix (ECM) additives. These can be incorporated during encapsulation by mixing the additive with cell culture media and resuspending the cell pellet before combining with the hydrogel.

Below are commonly used matrix components, recommended working concentrations, and expected effects on hydrogel stiffness.

Recombinant Laminins*

Laminin 111

Product: Biolaminin 111 LN (LN111)
Stock concentration: 100 µg/mL
Final concentration in hydrogel: 10 µg/mL
Method:

1. Mix LN111 and cell culture media (CCM) at a 1:1 ratio.
2. Resuspend the cell pellet in the LN111–CCM mixture.
3. Add 250 µL cell suspension per 1 mL hydrogel.

Impact on stiffness: None.

Laminin 521

Product: Biolaminin 521 LN (LN521)
Stock concentration: 100 µg/mL
Final concentration in hydrogel: 10 µg/mL
Method:

1. Mix LN521 and CCM at a 1:1 ratio.
2. Resuspend the cell pellet in the LN521–CCM mixture.
3. Add 250 µL cell suspension per 1 mL hydrogel.

Impact on stiffness: None.

* The addition of recombinant laminins can often be useful for culturing stem cells, particularly those which have been previously cultured with animal-derived hydrogels rich in laminin (such as those derived from EHS mouse sarcoma). The cells' dependence on laminin addition is often reduced or disappears after ~3 passages in this mixture.

Fibronectins

Human Fibronectin (from plasma)*

Product: Corning Fibronectin, Human
Stock concentration: 1 mg/mL
Final concentration in hydrogel: 40 µg/mL
Method:

- Mix fibronectin and CCM at a 1:4 ratio (50 µL FN + 200 µL CCM).
- Resuspend the cell pellet in this FN–CCM mixture.
- Add 250 µL cell suspension per 1 mL hydrogel.

Impact on stiffness: Typically reduces stiffness by one level (i.e., PMCORE125 + addition would have same stiffness as unmodified PMCORE100).

Bovine Fibronectin (from plasma)*

Product: STEMCELL Technologies Fibronectin
Stock concentration: 1 mg/mL
Final concentration in hydrogel: 40 µg/mL
Method:

- Mix fibronectin and CCM at a 1:4 ratio.
- Resuspend the cell pellet in the FN–CCM mixture.
- Add 250 µL cell suspension per 1 mL hydrogel.

Impact on stiffness: Typically reduces stiffness by one level (i.e., PMCORE125 + addition would have same stiffness as unmodified PMCORE100).

* Recombinant sources of fibronectin are likely not to have any impact on hydrogel stiffness at the same concentrations, due to lack of glycosylation in *E. coli* expression system.

Collagens

Collagen I (human)*

Product: CellAdhere Type I Collagen, Human
Stock concentration: 3 mg/mL
Final concentration in hydrogel: 100 to 200 µg/mL, depending on application
Method:

1. Calculate the required collagen stock volume. For example, 100 µg/mL final concentration requires 41.67 µL of stock per 1 mL hydrogel.

2. Neutralise the collagen using 1 M NaOH and adjust the total volume to 125 µL in 10X DPBS.
3. Mix neutralised collagen with CCM at a 1:1 ratio.
4. Resuspend the cell pellet in this Col–CCM mixture.
5. Add 250 µL cell suspension per 1 mL hydrogel.

Impact on stiffness: Typically reduces stiffness by one level (i.e., PMCORE125 + addition would have same stiffness as unmodified PMCORE100).

Collagen I (rat tail)*

Product: Gibco Collagen I, rat tail
Stock concentration: 3 mg/mL
Final concentration in hydrogel: 100 to 200 µg/mL
Method:
Same method as human collagen.
Impact on stiffness: Typically reduces stiffness by one level (i.e., PMCORE125 + addition would have same stiffness as unmodified PMCORE100).

* Recombinant sources of collagen are likely not to have any impact on hydrogel stiffness at the same concentrations, due to lack of glycosylation in *E. coli* expression system.

<u>Glycosaminoglycans (GAGs)</u>

Hyaluronic Acid (HA)

Product: Hyaluronic Acid Oligosaccharide dp8
Stock concentration: 1 mg/mL
Final concentration in hydrogel: 16 µg/mL
Method:

- For every 920 µL CCM, add 80 µL HA.
- Resuspend the cell pellet in the HA–CCM mixture.
- Add 250 µL cell suspension per 1 mL hydrogel.

Impact on stiffness: Undetermined.

Heparan Sulphate (HSIII)

Product: Heparan Sulphate fraction III
Stock concentration: 1 mg/mL

Final concentration in hydrogel: 16 µg/mL
Method:

- For every 920 µL CCM, add 80 µL HSIII.
- Resuspend the cell pellet in the HSIII–CCM mixture.
- Add 250 µL cell suspension per 1 mL hydrogel.

Impact on stiffness: Typically reduces stiffness by one level (i.e., PMCORE125 + addition would have same stiffness as unmodified PMCORE100).

Heparin (dp10)

Product: Heparin Oligosaccharide dp10
Stock concentration: 1 mg/mL
Final concentration in hydrogel: 16 µg/mL
Method:

- For every 920 µL CCM, add 80 µL heparin.
- Resuspend the cell pellet in the heparin–CCM mixture.
- Add 250 µL cell suspension per 1 mL hydrogel.

Impact on stiffness: Typically reduces stiffness by one level (i.e., PMCORE125 + addition would have same stiffness as unmodified PMCORE100).

8. DOCUMENT HISTORY

Version	Date	Summary of Changes
1.0	**05 Feb 26**	First version of customer facing SOP, adapted from internal PeptiMatrix cell encapsulation procedures.