PeptiMatrix™
Next generation peptide hydrogels

STANDARD OPERATING PROCEDURE

SEEDING CELLS ON 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™ Core (PMCORE125) diluted 1:1 with Phosphate Buffered Saline (e.g., DPBS)
- 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 seeding
- 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 diluted hydrogel to cell culture media** to ensure correct gelation.

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

1. Transfer 0.5 mL of PeptiMatrix hydrogel into a 15 mL centrifuge tube.
2. Add 0.5 mL of DPBS.
3. Vortex for 1 min.
4. 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.
2. Gels of lower concentration will become free flowing at this temperature.
3. Higher concentration gels may remain self-supporting but will be less viscous.
4. **Do not exceed 90 °C.** Higher temperatures can damage the gel.

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

1. For every 1 mL of diluted hydrogel, add 0.5 µL of 0.5M NaOH.
2. Vortex for 1 minute, then centrifuge to remove bubbles.
3. Visually inspect the gel. Repeat steps 1-2 as required to until gel becomes fully transparent again (typical recovery is 1-3 cycles).
4. 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 Mix cell culture media into the hydrogel

Mixing cell culture media into the hydrogel at this stage is important to ensure proper gelation.

For every 1 mL of diluted hydrogel, mix with 250 µL of cell culture media.

There are two methods commonly used for mixing cell culture media into the hydrogel. If your cell culture media contains any components sensitive to shear stress (such as any matrix additions you've decided to include – see below), we recommend **Option B**.

Option A

1. For every 1 mL of hydrogel, add 250 µL of cell culture media.
2. Vortex for 1 min.
3. Centrifuge at 600 x g for 1 minute to remove any trapped air bubbles.

Option B

1. Using a P1000 pipette, slowly dispense 250 µL of cell culture media 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.
4. Reverse pipetting (set to 200 µL) helps avoid air bubbles.
    a. For a description of reverse pipetting see **Section 6** below.
5. Once fully mixed, proceed to plating.

Optional: Adding soluble matrix components

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

1. Combine soluble matrix additions and cell culture 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**.

4.2 Plating the gels

1. Using a P200 pipette, dispense the hydrogel into a 96-well plate. Add at least 35 µL to the centre of each well. Complete coverage is important for consistent gelation.
2. Centrifuge plate at 600 x *g* for 1 minute to ensure even coverage.
3. Add up to 200 µL of media to each well of a dropwise on top of each plated gel.
4. Incubate the gels at 37 °C for 30 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. As gel thickness can influence cell behaviour, users may wish to optimise gel volume for their specific cell type and application. Suggested volumes are given below:

Plate size	Approx. volume per well
384-well plate	5 – 25 µL
96-well plate	35 – 50 µL
48-well plate	75 – 150 µL
24-well plate insert	75 – 150 µL
24-well plate	0.2 – 0.3 mL
12-well plate	0.3 – 0.5 mL
6-well plate	0.5 – 1 mL

4.2 Preparing the cell suspension

1. Detach and count cells using your standard protocol.
2. A good initial seeding density is between 10,000 and 40,000 cells per well of a 96-well plate, but may need to be optimised based on cell type and anticipated length of experiment.
3. Remove cell culture media from each well.
4. Add 200 µL cell suspension to each well.
5. 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.

Optional: Working with cells sensitive to pH

PeptiMatrix hydrogels are slightly alkaline before mixing with media. During mixing with cell culture media, 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, you may choose to perform one or two medium changes after the initial 30-minute incubation with cell culture medium, incubating for 30 minutes between changes, before adding the cell suspension. This can help the gel reach physiological pH prior to cell seeding

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 by combing with cell culture media before mixing 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. 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. 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).
- 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.
- 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. 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.
- 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.
- 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.
- 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 seeding procedures.