PeptiMatrix™
Next generation peptide hydrogels

STANDARD OPERATING PROCEDURE

EXTRACTING CELLS FROM 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 and eventually extracted from. 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. This process can also be reversed so that the gels undergo a gel-liquid transition, to extract cells for re-seeding or downstream processing.

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

- Class II microbiology safety cabinet (or appropriate class for your cell type)
- Centrifuge and 15 mL centrifuge tubes
- Microcentrifuge and 1.5 mL microcentrifuge tubes
- P1000, P200 pipettes and filter tips
- Cells encapsulated or seeded on PeptiMatrix hydrogels
- Cell culture media appropriate for your cells
- Dulbecco's Phosphate Buffered Saline (DPBS)

4. METHODS

4.1 Cell extraction from a 96-well plate

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

For reference, the steps below describe the process using cells encapsulated in **100 µL hydrogel** per well, plated into a **96-well plate**.

1. Using a P200 pipette, aspirate the old media on top of the gel from the well, removing ~75% of the media volume to minimise risk of disturbing the gel.
2. Using a P200 pipette, take up **200 µL** of new cell culture media/DPBS.
3. Insert the end of the pipette tip into the gel, until it is touching the **bottom** of the well plate.
4. Pipette up and down several times to **thoroughly mix** the cell culture media/DPBS with the gel. This will shear the gel and dilute it until it transitions to liquid phase.
    - For high stiffness hydrogels (PMCORE150), the gel might not completely transition back to liquid phase, but this can be addressed in the following step.
5. Transfer the total volume of the cell solution in the well to a 15 mL centrifuge tube **or** a 1.5mL microcentrifuge tube depending on how many total wells/what well plate size you are using. Repeat for as many other wells as required.
    - For high stiffness hydrogels (PMCORE150), the collected volume can now be further diluted with cell culture media/DPBS to ensure complete transition to liquid phase. Be sure to **thoroughly mix** any cell culture media/DPBS added to the collected volume in the same way as in the previous step.
6. The resulting solution can now be treated in the same way as a cell suspension of the cell type(s) in use. Cells can be pelleted at the centrifuge/microcentrifuge speed and duration they would normally be spun at with any other solutions.
7. The cell pellet should be visible at the bottom of the centrifuge/microcentrifuge tube, where the cells can be used for desired downstream processing. For example:
    - The cell pellet can then be re-suspended in fresh media for re-seeding/re-encapsulation (see separate SOP on Encapsulating Cells in PeptiMatrix hydrogels).
    - Pelleted cells can be combined with various commercially available lysis buffers to extract cell contents for downstream processing e.g., extraction of RNA for PCR analysis (see separate SOP on RNA extraction from cells in PeptiMatrix hydrogels).

A demonstration video of the above procedure is available on YouTube: *How to extract cells from PeptiMatrix hydrogels*

5. DISPOSAL

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

6. DOCUMENT HISTORY

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