Product Information Sheet

Peptide hydrogel matrix for cell culture

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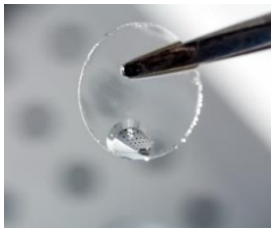

Peptide hydrogel matrix products for cell culture are designed to mimic extracellular matrix and more accurately reflect *in vivo* conditions. The products are created to meet the demands of

- ***3D at single cell and culture level.*** Higher elasticity and lower stiffness of hydrogels compared to cell culture plastic or insert membranes ensure bigger z-height, motility and communication of cultured cells. Hydrogels may be modified to ensure multilayering, increased cell-to-cell or cell-to-matrix interactions and spheroid or other 3D-shaped cultures depending on hydrogel matrix surface topography and/or biochemistry.
- ***Adaptation for cell type and experimental purpose.*** Customers may choose hydrogel components and the mode of microformation to design specific environment for their cells: introduce extracellular matrix components or selected motifs, activity modulators or guide the cells by microstructures.
- ***Ready to use, easy to handle.*** Hydrogel disks are shape-retaining and made in ready-to-use format.
- ***Compatibility with multiwell plates, membrane inserts and chamber slides.*** The matrices are made to fit all most popular labware formats but also can be custom-tailored for specific purposes.
- ***Visible cells at all stages.*** Hydrogel discs are made transparent to visualize cells at any point of the ongoing experiment or continuously, by direct and inverted, light and fluorescent microscopes.
- ***Freedom of manipulation.*** In contrast to multiwell plate bottom culture, cells on hydrogels can be easily taken out from the well and transfered to another environment if this is needed for your experimental procedure. For example, you can take out your cells from a 96 well plate and put into a bigger dish for patch clamping or combine several gels in co-culture vessel, lift on an insert for air-liquid interface cultures or stack several gels for specific tissue engineering purposes.

Characteristic		Value *
Composition	Basic	Water, collagen (type I) or collagen mimetic peptides (CMP) conjugated with polyethylene glycol (PEG)
	Case-specific	IKVAV, RGD, YGSR peptides, hyaluronic acid, or other bioactive compound
Water content, %		>90
Elastic (Young's) modulus		0.15-0.18
Elongation at break, %		58.30±5
Tensile strength (MPa)		0.07±0.1
Backscatter, %		<1
Light transmission, %		92.4 ± 0.1
Morphology		Fibrillar network, nanopores ~0.4 µm
Packaging		Ready to use hydrogels, encased separately, packed in vials with PBS (10mM, pH7,3). Shipped at ambient temperature.
Storage		2 - 6°C. Do not freeze.
Shelf life		6 months

Disk dimensions	Appropriate for multi-well plate
6 mmØ x 500µm	96 well plate (wp)
10 mmØ x0,5 mm	48, wp, 24 wp
15 mmØ x0,5 mm	24 wp
18 mmØ x0,5 mm	12 wp, 6 wp
On request	Custom specification

*Unless otherwise noted, all data provided for ~8% CMP (unmodified) basic concentration gels.

Directions for use

I. Preparation for use

STEP 1	In sterile environment, carefully place hydrogel discs into the wells where cells will be cultured.
STEP 2	Fill the wells with sterile PBS for 30 min. Carefully discard solution without dislodging the gel.
STEP 3	Repeat the procedure with fresh PBS.
STEP 4	Replace PBS with cell culture medium for 30 min. Aspirate and discard medium.
STEP 5	Seed the cells

II. Cautions

Do not freeze. Do not heat-sterilize or autoclave, nor subject to UV. All these procedures might cause structural changes to the hydrogel matrix and influence cell behavior.

III. Handling advice

- For hydrogel handling, use forceps with flat ends or flat spatulas to avoid gel damage.
- Pipetting should be done with care to avoid hydrogel damage. While aspirating carefully slide the pipette tip against the wall of the well gently sucking up the medium at the same time. Be careful aspirating close to the gel, try not to dislodge it.
- Antibiotics and/or antimycotics can be added at each step of washing.

IV. General recommendations for cell culture

There are no special requirements for cell preparation and seeding compared to usual procedures with commonly used culturware. If cell spheroid culture in micro-welled hydrogels is planned, care should be taken while calculating initial seeding density. Pilot experiments are recommended to establish an optimal cell seeding density to obtain a desired spheroid size.

V. Tuning the hydrogel for selected cells

- **For neural culture**

There are several ways to culture neuronal or neuroglial cells on Ferentis hydrogels. You can choose the appropriate modification dependent on your experimental purpose. Cells will form neurospheres of different size and shape on pure CMP, however, if you choose flat surface, bigger spheres over 50 mm diameter will flatten into semi-spheres. Generation of neurites on pure CMP will not be as intensive as that on hydrogel with IKVAV peptide modification. Microwell-formatted CMP hydrogel is best suited for production of isolated neurospheres of controlled size. IKVAV together with microwells will produce 3D organoids of similar size with intensive neurites organized into fiber-like structures.

- **For epithelial cultures**

For epithelial (skin, cornea, intestinal, airway etc.) cultures and epithelial differentiation hydrogels might be placed in air-liquid interface (ALI) and cultured for specific length of time. Use a conventional cell culture insert to maintain hydrogel-seeded cells in ALI. Refill cell culture medium as usual, approx. every second day. After differentiation, cell-seeded discs might be conveniently paraffin-embedded, sliced and subjected to various staining methods and immunohistochemistry.

VI. Immunocytochemistry or other staining

If cells on hydrogels are organized into spheroid-like structures they are more prone to detachment during numerous washing steps of immunocytochemistry procedure. To reduce the chances of cell detachment decrease your washing times if possible and perform all washing procedures with care. Alternatively you may cover the cells with hydrogel coverslips immediately after fixation step before starting immunocytochemistry. Such coverslips could be made from 3-4% agarose shed on a glass Petri dish as a thin 200-500 μm layer. When agarose gel is formed, place it on a lifted stage (such as external top of a Petri dish) and cut the coverslips out while pressing with the wells of the same size as your cells are cultivated in 96, 24, 6 well plate or other. Perforate the cutouts with a microneedle for better access of the reagents. Place the coverslips on hydrogel-cell cultures and keep them on top of your cells during the whole immunocytochemistry procedure. Take the coverslips out before microscopy. If making agarose coverslips seems complicated or time consuming, you can order ready to use Ferentis collagen hydrogel perforated coverslips for this purpose.

VII. Immunohistochemistry

Cryosectioning is not recommended for cell cultures on hydrogels because of high hydrogel water content: freezing water may disrupt hydrogel and cell structures on it. Paraffin embedding works well and does not influence cellular organization on hydrogels.

VIII. Microscopy

Because of high optical transparency cells can be visualized from both sides of the insertable matrices. For bigger magnifications with immersion, both fixed or live hydrogel-based cultures can be taken out from the multiwell plate and put on a microscope slide or glass-bottom dish. For better access of cells by inverted microscopy hydrogels can be placed cell side down. Avoid gell drying - keep the gel with the cells submerged in a buffer at all times and avoid trapped air bubbles between the hydrogel and the glass while placing the hydrogel for the microscopy. Use coverslip to minimize amount of buffer (excessive liquid allows hydrogel movements) and evaporation.

IX. Recovery of the cells or cell-content from the hydrogel

If cell suspension is needed for flow cytometry, other assays or reseeding - perform usual cell dissociation procedure (trypsin- or other enzyme-based) to recover the cells. In addition, cell lysis with lysing agents, DNA and/or RNA harvesting might be performed conventionally.