

Corning® PuraMatrix™ Peptide Hydrogel

Frequently Asked Questions

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Corning PuraMatrix Peptide Hydrogel (Cat. No. 354250) is a synthetic matrix that is used to create defined three-dimensional (3D) micro-environments for a variety of cell culture experiments. To achieve optimal cell growth and differentiation, it is necessary to determine the appropriate mixture of this material and bioactive molecules (e.g., growth factors, extracellular matrix [ECM] proteins, and/or other molecules). Corning PuraMatrix Peptide Hydrogel consists of standard amino acids (1% w/v) and 99% water. Under physiological conditions, the peptide component self-assembles into a 3D hydrogel that exhibits a nanometer scale fibrous structure. The hydrogel is readily formed in a Falcon® Cell Culture Dish, Plate, or Filter Permeable Support.

Cell Culture

What cell types/applications are suitable for this material?

Corning PuraMatrix Peptide Hydrogel has been shown to promote the differentiation of hepatocyte progenitor cells,¹ rat pheochromocytoma cells (PC12),² hippocampal neurons,³ and endothelial cells.⁴ Studies have also demonstrated that Corning PuraMatrix Peptide Hydrogel supports the attachment of a variety of primary (e.g., neuronal, fibroblast, keratinocyte) and transformed (e.g., MG-63, SH-SY5Y, HEK293, NIH3T3) cell types.^{5,6} Other potential applications include stem cell proliferation, tumor cell migration and invasion, angiogenesis assays, and *in vivo* analyses of tissue regeneration.

Does Corning PuraMatrix Peptide Hydrogel promote cell growth and differentiation in the absence of protein/growth factor supplementation?

To achieve optimal cell growth and differentiation, it is necessary to determine the appropriate mixture of Corning PuraMatrix Peptide Hydrogel and bioactive molecules (e.g., growth factors, ECM proteins, and/or other molecules).

Should cells be plated on top of the hydrogel (surface plating) or encapsulated within the material (3D encapsulation protocol)?

Either option is possible. The optimal plating configuration will depend on the cell type and the experimental objectives. Please refer to the Corning Guidelines for Use for specific examples and recommendations.

If cells are seeding onto the material using the surface plating method, will the cells migrate into the hydrogel?

Since the hydrogel 'fibers' are flexible (not covalently linked), cells can migrate into the hydrogel in some cases. The capacity for migratory behavior will be dependent on the cell type used.

What seeding density should be used?

We suggest a seeding density comparable to that used for surface plating on standard tissue culture-treated or ECM-coated substrates. A seeding density of 0.5-1.0 x 10⁶ cells/mL is recommended for 3D encapsulation cultures.

How long can cells survive on Corning PuraMatrix Peptide Hydrogel?

The actual duration of the culture will depend on the cell type and culture conditions. In some cases, cells can be maintained for as long as six weeks following 3D encapsulation or for about one to two weeks following surface plating on the hydrogel.

Can I sub-culture cells that have been maintained on Corning PuraMatrix Peptide Hydrogel?

Yes. Cells can be recovered from the hydrogel and then sub-cultured for an additional period of growth/differentiation using a fresh layer of Corning PuraMatrix Peptide Hydrogel or an alternative growth substrate (e.g., TC-treated, ECM-coated). Please refer to the Corning Guidelines for Use for the cell recovery protocol.

References

1. Semino, C.E., et al., Functional differentiation of hepatocyte-like spheroid structures from putative liver progenitor cells in three-dimensional peptide scaffolds. *Differentiation* 71:262 (2003).
2. Holmes, T.C., et al., Extensive neurite outgrowth and active synapse formation on self-assembling peptide scaffolds. *PNAS USA* 97:6728 (2000).
3. Semino, C.E., et al., Entrapment of migrating hippocampal neural cells in 3D peptide nanofiber scaffold. *Tissue Engineering* 10:643 (2004).
4. Narmoneva, D., et al., Self-assembling short oligopeptides and the promotion of angiogenesis. *Biomaterials* 26:4837 (2005).
5. Zhang, S., et al., Self-complementary oligopeptide matrices support mammalian cell attachment. *Biomaterials* 16:1385 (1995).
6. Thonhoff, J.R., et al., Compatibility of human fetal neural stem cells with hydrogel biomaterials *in vitro*. *Brain Research* 1187:42 (2008).

Material Properties and Handling

Why is it necessary to work quickly when mixing cells with this material prior to gelation?

The stock solution of Corning® PuraMatrix™ Peptide Hydrogel (1% w/v) exhibits a pH at 3.0, which can adversely affect cell viability. Therefore, it is important to work quickly to minimize the amount of time that cells are in contact with this material prior to the addition of culture medium. The culture medium is changed three times within the first 30 minutes to equilibrate the sample to physiological pH.

What is the mechanical strength of Corning PuraMatrix Peptide Hydrogel?

At the typical working concentration (0.5% w/v), the hydrogel forms a soft fibrous network that exhibits a relatively weak mechanical strength. Therefore, it is necessary to handle the material very carefully when performing medium changes (i.e., avoid direct contact with the hydrogel when using pipet or aspiration tips). Importantly, the 0.5% hydrogel has been found to promote the attachment and growth of many cell types. To prepare a hydrogel with greater mechanical strength, the material can be used in the undiluted form (1% w/v).

What is the best way to remove air bubbles that result from mixing the material with cells, bioactive molecules, and/or medium?

Air bubbles can be removed by centrifugation (e.g., spin two to five minutes at 5K in a tabletop centrifuge). If small volumes are used in a 1.5 mL eppendorf tube, centrifugation can be performed using an Eppendorf microfuge for 10-15 seconds at full speed.

Can Corning PuraMatrix Peptide Hydrogel be used for *in vivo* studies in animals?

Yes. The soluble material can be injected and will subsequently form a 3D hydrogel upon contact with the physiological environment. Corning PuraMatrix Peptide Hydrogel can be readily handled using small/large bore needles and catheters. To avoid the introduction of bubbles *in vivo*, use extreme care when filling and injecting samples with needles that are smaller than 20G.

Since the peptide sequence of Corning PuraMatrix Peptide Hydrogel is similar to RGD, do cells bind to the material via RGD-dependent integrin receptors?

No. The peptide sequence promotes cell attachment, but does not mediate RGD-dependent integrin signaling. Studies have shown that cell attachment is not inhibited by RGD-containing peptides.⁵

Analytical Studies

Is Corning PuraMatrix Peptide Hydrogel compatible with confocal microscopy?

Yes. This material forms a clear gel that allows good resolution for microscopy applications.

Can I perform molecular analyses of proteins and nucleic acids using cells cultured in Corning PuraMatrix Peptide Hydrogel?

Yes. TCells cultured in the hydrogel can be prepared for most molecular techniques. Following mechanical disruption of the gel, the cells can be isolated using centrifugation and then processed according to standard procedures.

Is Corning PuraMatrix Peptide Hydrogel compatible with staining and immunodetection protocols?

Yes. Cells can be stained with fluorescent dyes or immunological reagents using standard methods.

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