

Directions for Use

PhotoGel®-IRG Methacrylated Gelatin Kit

GELATIN METHACRYLATE WITH IRGACURE KIT FOR UV-CROSSLINKABLE HYDROGELS
Catalog Number #5215-1KIT

Product Description

Three dimensional (3D) gels allow for the study of the effects of the mechanical properties of the extracellular matrix (ECM), such as density and rigidity, on cell development, migration, and morphology. Unlike 2D systems, 3D environments allow cell extensions to simultaneously interact with integrins on all cell surfaces, resulting in the activation of specific signaling pathways. Gel stiffness or rigidity also affects cell migration differently in 3D versus 2D environments.

Furthermore, integrin-independent mechanical interactions resulting from the entanglement of matrix fibrils with cell extensions are possible in 3D systems, but not in 2D systems where the cells are attached to a flat surface.

Advanced BioMatrix offers PhotoGel®, a purified gelatin methacrylate kit, which provides native-like 3D gelatin gels with the unique attributes to be prepared at various concentrations and UV-crosslinked to provide various gel stiffness. Though it is denatured collagen, gelatin retains many natural binding motifs such as RGD and MMP sites.

The PhotoGel® UV-kit consists of gelatin methacrylate and a UV-activated photoinitiator.

Table 1:

Item	Catalog No.	Package Size
Methacrylated Gelatin, Lyophilized	5208	2 X 500 mg (Total 1 gram)
Photoinitiator	5200-100MG	100 mg

Gelatin Methacrylate is produced from porcine, type A, 300 bloom gelatin. Gelatin macromers containing primary amino groups were reacted with methacrylic anhydride (MA) to add methacrylate pendant groups.

Our Gelatin Methacrylate achieves a degree of substitution of >70% for maximum crosslinkability and range of stiffness.

The photoinitiator solution consists of Irgacure 2959 which needs to be formulated in methanol (not included) allowing for UV crosslinking of the gelatin at 365 nm.

Characterization and Testing

The formulated PhotoGel® has the following characteristics as shown in Table 2.

Table 2:

Test	Specifications
Cell Compatibility	>70% viability
Grafting Efficiency	≥75%
Sterility	No growth

Storage/Stability:

The product ships on frozen gel packs. Upon receipt, store the gelatin methacrylate at -20°C. Store the Irgacure at 2 to 10°C

Preparation Instructions

Note: Employ aseptic practices to maintain the sterility of the product throughout the preparation and handling of the collagen and other solutions.

Note: The following instructions are for a 10% gelatin methacrylate solution. Recommended concentrations are 5-20%

1. Warm 10 mL of sterile warm 1X PBS or 1X cell culture media to >60°C.
2. Add the 5 mL's of warmed solution to the amber vial containing 500 mg of lyophilized gelatin methacrylate.
3. Mix on a shaker table or rotator plate until fully solubilized. Keep warm (>37°C) if possible (eg. place your rotator in an incubator) to help with full

solubilization.

4. Add 1 mL of neat methanol to the small amber vial of photoinitiator containing 100 mg of Irgacure, and vortex.
(Note – Irgacure *in solution* has a shelf life of 2 weeks. Only dissolve required amount of Irgacure powder in a 10% solution)
5. Calculate the volume of the photoinitiator required by multiplying the total volume of gelatin required by 0.01. (If you want to add it to the 5 mL directly, you would add 0.05 mL of irgacure)
6. Add the calculated volume of photoinitiator to the required volume of gelatin solution and mix thoroughly.
7. Add your cells to the gelatin/photoinitiator solution.
8. Dispense your gelatin/photoinitiator/cell solution into the desired dish (ie. 6-well plate, 48-well plate).
9. For UV-crosslinking, place printed structure directly under a 365 nm UV light crosslinking source.

Longer exposure allows more crosslinking, though each cell type withstands different degrees of UV light and free radicals (generated by the photoinitiator) that mediate crosslinking.

Any excess material can be refrigerated and stored. The material will gel. Warm back up to >30°C for it to become liquid again.