Directions for Use
PhotoHA® -LAP Methacrylated Hyaluronic Acid Kit

HYALURONIC ACID METHACRYLATE WITH LAP KIT FOR UV-CROSSLINKABLE HYDROGELS

Catalog Number **#5274-1KIT**

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.

Hyaluronic acid is the most abundant glycosaminoglycan in the body being an important component of several tissues throughout the body. While it is abundant in extracellular matrices, hyaluronan also contributes to tissue hydrodynamics, movement and proliferation of cells, and participates in a number of cell surface receptor interactions.

Hyaluronic acid is a polymer of disaccharides, themselves composed of D-glucuronic acid and *N*-acetyl-D-glucosamine, linked via alternating β-(1→4) and β-(1→3) glycosidic bonds. Hyaluronic acid can be 25,000 disaccharide repeats in length. Polymers of hyaluronic acid can range in size from 5,000 to 20,000,000 Da *in vivo*.

Hyaluronic acid is energetically stable, in part because of the stereochemistry of its component disaccharides. Bulky groups on each sugar molecule are in sterically favored positions, whereas the smaller hydrogens assume the less-favorable axial positions.

Advanced BioMatrix offers PhotoHA®, a purified hyaluronic acid (HA) methacrylate kit, which provides native-like 3D HA gels with the unique attributes to be prepared at various concentrations and photocrosslinked to provide various gel stiffness.

Hyaluronic acid contains primary amino groups which are reacted with methacrylic anhydride (MA) to add methacrylate pendant groups to the hyaluronic acid molecule. The method renders the hyaluronic acid into a product with unique properties.

The PhotoHA® LAP-kit consists of HA methacrylate and a photoinitiator.

Table 1:

Item	Catalog No.	Package Size
Methacrylated Hyaluronic Acid, Lyophilized	5212-100MG	100 mg
Photoinitiator LAP	5269-100MG	100 mg

The photoinitiator solution consists of LAP which needs to be formulated in 1X PBS or cell culture media, allowing for photocrosslinking of the PhotoHA® at 405 nm.

Characterization and Testing

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

Table 2:

Test	Specifications
pH (when solubilized with PBS)	6.0 – 8.0
Osmolality (when solubilized with PBS)	200 to 400 mOsmo/Kg H_2O
Molecular Weight of Hyaluronic Acid	100-150 kDa
NMR Analysis	Characteristic
Cell Compatibility	Characteristic
Grafting Efficiency (Uronic Acid method)	Provided on the C of A for each lot.
Sterility	No growth

Storage/Stability:

The product ships on frozen gel packs. Upon receipt, store the PhotoHA® at -20°C. Store the LAP at 2 to 10°C. The product and components are stable for a minimum of 1 year at receipt in powder form. Once solubilized, the PhotoHA® can be stored at 2-10°C for 1 month. The photoinitiator can be stored for no more than 2 weeks once solubilized.

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: For the majority of cells, it is recommended to add additional proteins (ie. collagen) to the hyaluronic acid hydrogel, to provide cell binding sites.

Recommended concentrations are 5-30 mg/ml (0.5-3.0%).

Note: The following recommended instructions are for a 1% hyaluronic acid (HA) methacrylate solution. Adjustments to this protocol may be required for various concentrations.

1. Add 10 ml of 1X phosphate buffer saline (PBS), water or cell culture media to the 100 mg of lyophilized methacrylated HA powder.

2. Mix on a shaker table or rotator plate until fully solubilized (~30 to 60 minutes) at 2-10°C.

   Note: Solubilization times may vary depending on the desired concentration and volume of PBS, water or medium added,

3. Calculate the volume of photoinitiator to add by multiplying the volume of solubilized hyaluronic acid by 0.02. If the resulting number is 200 ul, for example, you will add 200 ul of LAP.

4. Solubilize the required amount of LAP (per step 3) at a concentration of 17 mg/ml in 1X PBS or cell culture media.

5. Add the calculated volume of photoinitiator to the required volume of HA methacrylate solution and mix thoroughly.

6. Add your cells to the HA methacrylate /photoinitiator solution.

7. Dispense your HA methacrylate /photoinitiator/cell solution into the desired cultureware (i.e. 6-well plate, 48-well plate).

8. For photocrosslinking, place the hydrogel solution directly under a 405 nm light crosslinking source.