DIRECTIONS FOR USE

Sterile Ready-to-Use Gelatin Methacrylate (GelMA) Solution Ink

This is a suggested procedure, please adjust according to your experimental needs.
To maintain the sterility of the product, work under sterile conditions.
For Research Use Only. Not for use in diagnostic or therapeutic procedures**.** Not for use in humans.

Manufactured by Advanced BioMatrix.

Storage/Shelf Life

This product ships with a cold pack. Upon receipt, store the GelMA solution ink at 2 to 8 °C. This product should be kept from moisture and light (including ambient light).

Concentration

The concentrations of GelMA and photoinitiator (LAP) are marked on the label of the product packages.

Bioprinting Protocol

The aim of this protocol is to provide instructions for bioprinting with the GelMA solution ink using the BIONOVA X 3D bioprinter with or without cells. This is an example procedure that may be adjusted accordingly to the intended application.

1. Remove the GelMA solution ink from the 2 to 8 °C refrigerator, warm the GelMA solution ink to 37 °C, and gently shake well to mix it homogeneously before use (avoid air bubbles).

***Note 1:**

- DO NOT Vortex the GelMA solution.

2. Bioprinting with cells: (skip this step and move to Step 3 if printing without cells)

Prepare the final solution ink for bioprinting by gently mixing (avoid air bubbles) the provided GelMA solution ink with desired volume of high-density cell suspension in media to achieve the desired **final concentrations (see Note 2) of GelMA, LAP, and cells**.

***Note 2:**

- Recommended **final concentration** of GelMA solution is 50 - 100 mg/ml (5.0 - 10% w/v).
- Recommended **final concentration** of LAP photoinitiator solution is 1.25 – 2.5 mg/ml (0.125 – 0.25% w/v).
- Recommended **final concentration** of cells is 1 million/ml – 20 million/ml.

3. Bioprinting without cells:

Prepare the final solution ink for bioprinting by mixing the provided GelMA solution ink with desired buffers (i.e. 1X DPBS) to achieve the desired **final concentrations (see Note 3) of GelMA and LAP**.

***Note 3:**

- Recommended **final concentration** of GelMA solution is 50 - 100 mg/ml (5.0 - 10% w/v).

- Recommended **final concentration** of LAP photoinitiator solution is 1.25 – 2.5 mg/ml (0.125 – 0.25% w/v).

4. Bioprinting on BIONOVA X bioprinter:

4.1 Power on the BIONOVA X bioprinter, sterilize the printer for desired time when the sliding door of the printer is closed.

4.2 Once the UV sterilization process is completed, open the sliding door and load the sterile BIONOVA X printing probe.

4.3 Load proper volume of the final solution ink to a sterile BIONOVA X multi-well plate by using a pipette.

***Note 4:**

Choose the printing probe to match the desired multi-well plate. The volume of the bioink should not exceed the maximum volume suggested in the following table:

Printing probe and well plate used	6-well probe and 6-well plate	12-well probe and 12-well plate	24-well probe and 24-well plate
Maximum volume of bioink for each well (ml)	8.6	3.4	1.3

4.4 Load the well plate onto the plate tray of BIONOVA X bioprinter.

4.5 Load a previously saved printing Project or create a new Project to print the desired scaffold.

***Note 5:**

- When printing with GelMA bioink, the recommended printing temperature is 37-40 °C, the recommended printing motion speed is 0.005-0.05 mm/s.
- When printing with cells, the mixing function is recommended to be utilized.
- The light exposure conditions should be adjusted accordingly to your own application. Recommended light intensity is 50%-100%.
- In general, the crosslinking density of the hydrogels will increase with higher light intensity, longer exposure time, and higher concentrations of bioink and photoinitiator. Increased crosslinking density will result in a stiffer hydrogel.
- Please refer to the Manual of BIONOVA X for more information regarding the printing setup.

5. After the bioprinting:

5.1 Open the sliding door and carefully remove the well plate from the plate tray of BIONOVA X.

5.2 Gently remove the uncured bioink solution from the wells and gently rinse the bioprinted samples with desired buffer solution or cell culture medium. The bioprinted samples are ready for culturing, imaging or assaying.

***Note 6:**

- Warm buffer or medium are recommended to wash the scaffolds printed by GelMA solution ink.