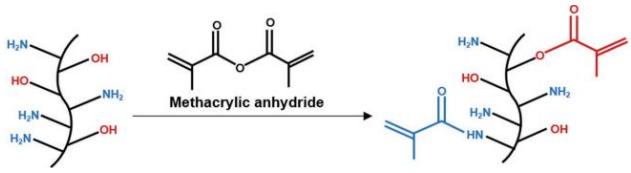
Instructions for use

hPLMA - PHOTOPOLYMERIZABLE HYDROGEL

Human platelets source

hPLMA is manufactured from platelet units obtained from screened healthy donors designated for therapeutic transfusion. Each donor undergoes testing, and non-reactivity is confirmed for HBsAg, anti-HBc, anti-HIV-1/2, anti-HCV, anti-HTLV-1/2, anti-T. cruzi, HIV-1, HCV, HBV, WNV nucleic acid testing, and syphilis micro hemagglutination assay. It is essential to observe universal precautions for handling and disposing of biological products while working with hPLMA.

hPLMA is chemically modified with photoresponsive groups to polymerize only when exposed to ultraviolet (UV) or visible light.



hPL hPLMA

Therefore, a photoinitiator is required to initiate the polymerization process **(not provided).** In Table 1, you will find a list of recommended photoinitiators for your reference.

Table 1. Photoinitiators and their respective wavelength compliance.

PHOTOINITIATOR	
Irgacure 2959	UV-Light (300nm)
LAP	Blue Light (405nm)

Uses: 3D cell culture, tissue engineering, disease modelling, and cancer and stem cell research.

hPLMA was designed to facilitate your cell culture!

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1. Reconstitution protocol

1. Considering your working volumes (V_w), you can use Equation 1 to accurately determine the required mass of hPLMA. In this equation, C_{hPLMA} represents the desired hPLMA concentration (w/v), m_{hPLMA} is the amount of hPLMA needed, and V_w is the working volume. Consult Table 2 for some suggested working volume options.

Note: The recommended concentration is between 10 - 25% w/v.

$$\textbf{Equation 1: } m_{hPLMA} = V_w \times C_{hPLMA}$$

Table 2. Options for Working Volumes Depending on the Well-Plate Type.

WELL PLATE	Volume of Hydrogel (µL)	Volume of Cover Medium (µL)
6 well plate	1000 - 1500	1000 - 3000
12 well plate	500 - 700	1000 - 2000
24 well plate	300 - 350	500 - 1000
48 well plate	100 - 150	200 - 400
96 well plate	30 - 50	100 - 200

2. Using sterile tweezers or a microspoon spatula, weigh the hPLMA into a sterile centrifuge tube to obtain the required amount according to Equation 1.

Example: Our required volume is 100 µL of hPLMA with a concentration of 15% (w/v). The corresponding weight of hPLMA needed is 15 mg.

3. Weigh the photoinitiator according to the working volume (V_w). The recommended concentration for the photoinitiator in the solution is 0.5% (w/v). **Please note that the photoinitiator may not be sterile**. When preparing the volume for your solution, make sure to prepare an excess volume for filtration purposes (usually ~400 µL is the retained-solution volume considering a syringe filter Ø30mm and 0,2µm pore size).

Example: The required volume is 100 µL, and since the photoinitiator is not sterile (e.g LAP), it is necessary to prepare 500 µL of a 0.5% LAP solution (100 µL working volume + 400 µL for filtration losses).

4. Dissolve the photoinitiator in a saline solution (e.g.: PBS or DPBS). We recommend using a vortex during this step.

Note: Do not use water to dissolve the photoinitiator.

5. Filter the photoinitiator solution, using a sterile syringe filter, into a sterile centrifuge tube.

6. Pipette the sterile working volume of the photoinitiator solution (V_w) into a centrifuge tube with hPLMA. Mix well by gently vortexing to avoid bubble formation.

2.1 Instructions for 2D hydrogel coating

1. Prepare cell cultures as recommended by the cell supplier to establish a stable population.

2. Reconstitute hPLMA lyophilizate according to the Reconstitution protocol in section 1.

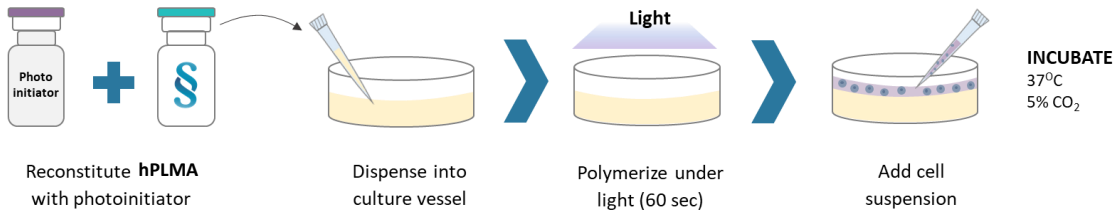
3. Add sufficient volume of hPLMA to completely cover the growth surface. If needed, discard the excess of the cover hPLMA solution.
   Eliminate any air bubbles that may have formed with the aid of a sterile needle.

4. Polymerize by exposing to an adequate light source for an adequate exposure time. **Please, pay attention to the light wavelength of your light source. Ensure that you use the right source according to your chosen photoinitiator.**

   Example: The recommended exposure time for a volume of 50 µL, when working with LAP, is ~60 seconds. If you are working with a higher volume, please adjust the exposure time accordingly.

5. Harvest cells from the culture and pipette the desired cell density to the top of the hydrogel.

   Note: Cell density may vary by cell type and assay purpose. Please consult the literature or cell supplier to identify the best cell density.



2.2 Instructions for 3D hydrogel encapsulation

1. Prepare cell cultures as recommended by the cell supplier to establish a stable population.

2. Reconstitute hPLMA lyophilizate according to the Reconstitute protocol in section 1.

3. Harvest cells from the culture and centrifuge the desired cell density.

§ **Note:** Cell density may vary by cell type and assay purpose. Please consult the literature or cell provider to identify the best cell density.

4. Centrifuge the amount of cell needed for the total number of conditions and gel volume previously established.

5. Remove **all the supernatant** from the cell suspension until you obtain a dry cell pellet.

Note: If you don't remove all the supernatant, it will affect the final hPLMA solution concentration.

6. Resuspend the cell pellet in hPLMA solution and mix by pipetting.

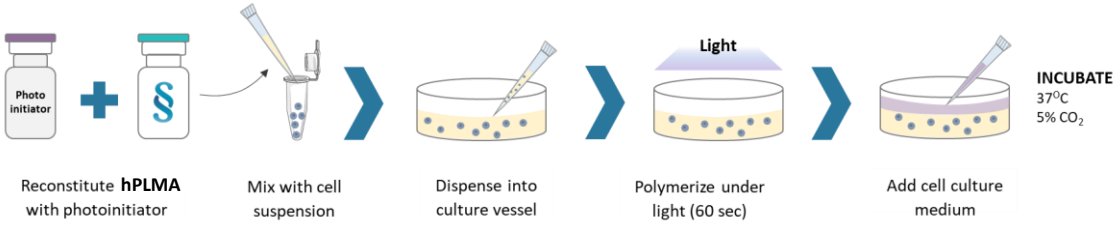
7. Pipette cell suspension to an adequate culture platform (see Table 2 for well-plate volume suggestions).

8. Polymerize by exposing to an adequate light source for an adequate time. **Please, pay attention to the light wavelength of your light source. Ensure that you use the right source according to your photoinitiator.**

§ **Example:** The recommended exposure time for a volume of 50 µL is 60 seconds. If you are working with a higher/lower volume, please adjust the exposed time accordingly.

After the exposed time, confirm that you have a hydrogel!

9. Add adequate volume of pre-warmed cell culture media (see Table 2 for volume recommendations) and place the culture platform in the incubator with the appropriate conditions for cell culturing (e.g. 37°C in a humidified environment with 5% CO2).



3.0 Suppliers

Table 3. List of reagents and equipment, considering the supplier and corresponding catalog number details.

Reagents/ Equipment	Supplier	Catalogue Number
Irgacure 2959	Sigma-Aldrich	410896
LAP	Biosynth Carbosynth Sigma-Aldrich	FL 146250 900889-0001
UV Lamp - Valo Cordless Ultradent (LAP)	Henry Schein	901-4087
UV lamp for OmniCure S2000	Sarspec	012-64000R