Instructions for use

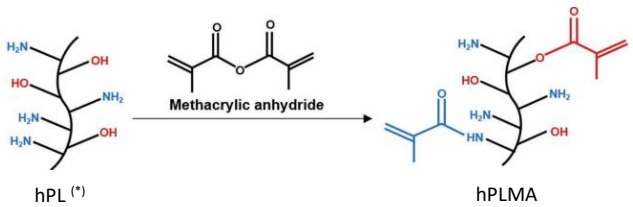
hPLMA – HUMAN METHACRYLOYL PLATELET LYSATES

Catalogue Number: PL01

Human platelets source

hPLMA-Human Methacryloyl Platelet Lysates 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: Human platelet lysates.

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 already tested and their respective wavelength compliance.

PHOTOINITIATOR	
LAP (Lithium phenyl-2,4,6-trimethylbenzoylphosphinate	Blue Light (405nm)
Irgacure 2959	UV-Light (300nm)

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 mass of hPLMA needed, and V_w is the working volume. Consult Table 2 for some suggested working volume options.

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

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

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 microspoon spatula, **mix well hPLMA podwer before use**.
3. Using sterile tweezers, transfer and weigh the required amount of hPLMA into a sterile container, according to the calculation described in Equation 1.

Example: For a final volume of **100 µL** and an hPLMA concentration of **15% (w/v)**, the required amount of hPLMA is **15 mg**.

After the initial opening, always seal the hPLMA vial lid with parafilm to protect it from moisture. If possible, store the vial in a desiccator for additional protection.

4. Weigh the photoinitiator according to the working volume (V_w). The recommended concentration range of LAP in the solution is 0.2-0.5% (w/v) and may be adjusted depending on the cell type and the specific experimental objective. **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: If the required volume is 100 µL, and if the photoinitiator (e.g. LAP) is not sterile it is necessary to prepare 500 µL of a 0.5% LAP solution (100 µL working volume + 400 µL for filtration losses).

For guidance on selecting the appropriate hPLMA and photoinitiator concentrations, please consult the hPLMA Powder Product Data Sheet or contact our Customer Support team.

5. Dissolve the photoinitiator in a saline solution (e.g.: PBS, DPBS or cell culture medium). We recommend using a vortex during this step.

Note: Do not use water to dissolve the photoinitiator.

6. Filter the photoinitiator solution into a sterile container using a sterile syringe filter.

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

2.1 Instructions for 2D hydrogel coating

1. Prepare cell cultures according to the cell supplier's recommendations to establish a stable population.

2. Reconstitute hPLMA lyophilizate according to the protocol described in Section 1 to obtain the working volume (V_w) needed of precursor hPLMA solution.

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

4. Polymerize the hydrogel by exposing it to an appropriate light source for the recommended exposure time. **Please, ensure that the light wavelength is compatible with the photoinitiator used, see Table 1.**

> **Example:** The recommended exposure time for a volume of 50 µL, when working with LAP, is 40-60 seconds. If you are working with a different volume, please adjust the exposure time accordingly.

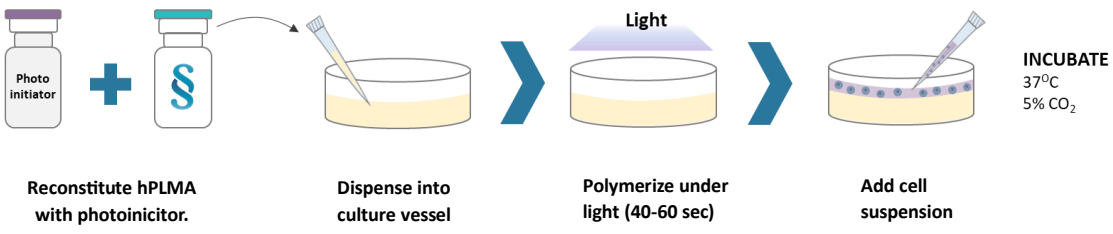
5. Harvest cells from the culture and count them to determine the current cell density.

6. Calculate the volume of cell suspension needed to achieve 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.

7. Seed the desired cell suspension volume onto the top of the polymerized hydrogel.

8. 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).



2.2 Instructions for 3D hydrogel encapsulation

1. Prepare cell cultures according to the cell supplier's recommendations to establish a stable population.

2. Reconstitute hPLMA lyophilizate according to the protocol described in Section 1 to obtain the working volume (V_w) needed.

3. Harvest cells from the culture and count them to determine the current cell density.

4. Calculate the volume of cell suspension needed to achieve the desired cell density and centrifuge this volume.

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

5. Carefully remove **all the supernatant** from the cell suspension pellet.

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

6. Resuspend the cell pellet in the precursor hPLMA solution (prepared according to the Section 1. protocol) and mix by pipetting until homogeneous.

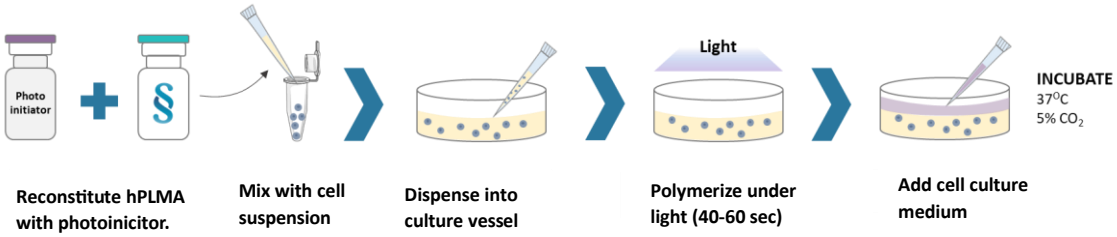
7. Pipette the precursor hPLMA solution containing the cells into an adequate culture platform (see Table 2 for well-plate volume suggestions) or into Polydimethylsiloxane (PDMS) molds (e.g. 2, 5 or 6-mm diameter and 2 mm of height).

8. Polymerize the hydrogel by exposing it to an appropriate light source for the recommended exposure time. **Please, ensure that the light wavelength is compatible with the photoinitiator used. See Table 1.**

§ **Example:** The recommended exposure time for a volume of 50 µL is 40- 60 seconds. If you are working with a different 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. Suppliers

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

Reagents/ Equipment	Supplier	Catalogue Number
LAP	Metatissue	PH01
UV Lamp - Valo Cordless Ultradent (for LAP)	Henry Schein	901-4087
UV Lamp - OmniCure S2000 (for Irgacure)	Sarspec	012-64000R

Contact us

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