Instructions for use

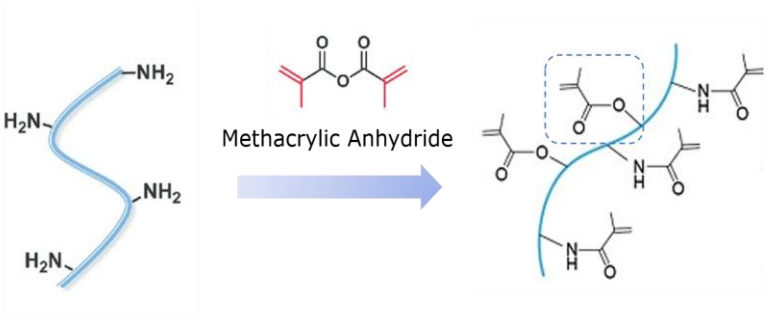
hPCMA – Human Methacryloyl Placenta

Catalogue Number: PC01

Placentas source

hPCMA - Human Methacryloyl Placenta is manufactured from human placentas (hPCs) obtained from licensed healthcare establishments after the donor's informed consent. All placenta donations are compliant with the Convention on Human Rights and Biomedicine, and Data Protection Regulation.
It is essential to observe universal precautions for handling and disposing of biological products while working with hPCMA.

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



Lyophilized dPC (*) hPCMA

(*) dPC: Decellularized Placenta.

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. Photoinitiator already tested and its respective wavelength compliance.

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

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

hPCMA was designed to facilitate your cell culture!

Index

1. Reconstitution protocol

Note: Prepare the precursor hPCMA solution 24 hours before the Cell Culture Assay and dissolve it at 4 – 15 °C with continuous shaking.

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

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

Note: The recommended concentration for hPCMA solutions is between: 0.7 – 1.5% (w/v) or 7 – 15 mg.mL^{-1}

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 spatula, weigh the hPCMA into a sterile container to obtain the required amount according to Equation 1.

Example: Our required volume is 100 µL of hPCMA solution with a concentration of 1% (w/v). The corresponding weight of hPCMA needed is 1,0 mg.

$$\textbf{Equation 1: } m_{hPCMA} = 0{,}100\ mL \times 10\ mg.mL^{-1}$$
$$m_{hPCMA} = 1.0\ mg$$

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

3. Weigh the photoinitiator according to the working volume (V_w). The recommended concentration of LAP in the solution ranges from 0.02% (w/v) to 0.1% (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 Ø30 mm and 0,2 µm pore size).

   § **Example:** 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 0.1% LAP solution (100 µL working volume + 400 µL for filtration losses).

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

   Note: Do not use water to dissolve the photoinitiator.

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

6. Pipette the sterile working volume of the photoinitiator solution (V_w) into a sterile container with lyophilized hPCMA.

7. Dissolve the precursor hydrogel solution at 4–15°C overnight or until the lyophilized hPCMA is fully dissolved. Use a thermomixer (shake at 1000 rpm). For volumes greater than 5 mL or if dissolution is challenging, adjusting the solution's pH to 7.4–7.6 is recommended.

8. After complete dissolution, keep the precursor hPCMA solution between 4 – 15ºC until polymerization. For storage periods longer than 1 day, keep the precursor hPCMA solution at −20 °C to prevent biochemical alterations.

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 lyophilized hPCMA according to the protocol described in Section 1 to obtain the working volume (V_w) needed of precursor hPCMA solution.

3. Add sufficient volume of precursor hPCMA 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 or photoinitiator, please adjust the exposure time accordingly.

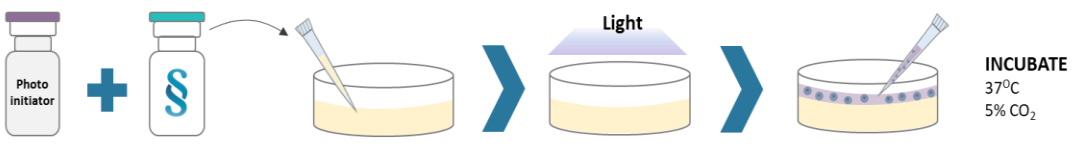
5. Harvest cells from the culture and count them to determine the current cell density.

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% CO_2).



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 lyophilized hPCMA 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 hPCMA solution concentration.

6. Resuspend the cell pellet in the precursor hPCMA solution and mix by pipetting until homogeneous.

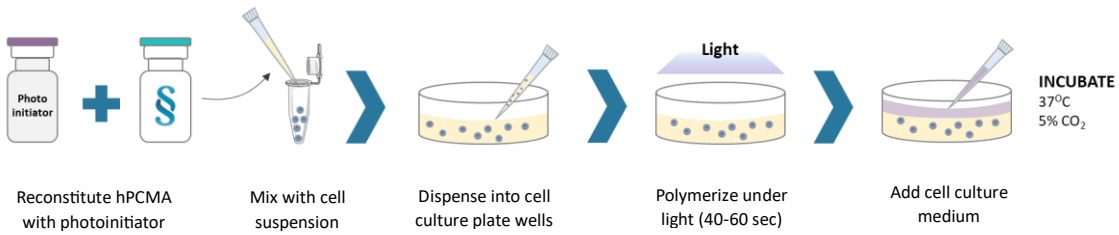
7. Pipette the precursor hPCMA solution containing the cells into an adequate culture platform (see Table 2 for well-plate volume suggestions) or into Polydimethylsiloxane (PDMS) molds (e.g. 2, 5 or 6-mm diameter and 2 mm of height).

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 when working with LAP is 40-60 seconds. If you are working with a different volume or photoinitiator, 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% CO_2).



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

Contact us

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