Instructions for use

hPLMA – Hydrogels Cell Recovery

Cell Recovery from Hydrogels

Efficiently recovering encapsulated cells from hydrogels is crucial in tissue engineering and regenerative medicine. Hydrogels derived from human proteins, such as Platelet Lysate Methacrylated (hPLMA), provide an excellent platform for 3D cell culture due to their bioactivity and customizable properties. Ensuring effective cell recovery from these hydrogels is vital for downstream applications, including cell viability assessments, proliferation studies, and other cell biology processes.

Enzymatic degradation is a widely used method for dissociating cells from hydrogels. Pronase, a proteolytic enzyme mixture with broad substrate specificity, is frequently employed for this purpose due to its ability to break down extracellular matrix components without significantly harming the encapsulated cells.

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1. Materials and Reagents

- Proteolytic enzyme (e.g. Pronase or collagenase IV).
- PBS (Phosphate Buffered Saline).
- 0.2 µm Syringe Filter.
- Incubator (37°C, 5% CO2).
- Centrifuge.
- Microcentrifuge Tubes.
- Pipettes and Tips.

Note: This protocol was prepared considering standard conditions performed in a 96 well plate using Pronase or collagenase IV. Any alteration needs to be validated and optimized accordingly.

2. Enzyme preparation

1. Determine the amount of enzyme needed to dissolve all the hydrogels. Consider 100 µl of enzyme solution for each hydrogel.

2. Dissolve the enzyme in PBS at the desired concentration (e.g. pronase: 0.05–0.25 (%w/v), collagenase IV: 0.5-1% (%w/v)).

Note: Enzymes concentration might have to be optimized according to the hydrogels rigidity and size.

Table 1. Degradation times for standard conditions

hPLMA		20µL Hydrogels			50µL Hydrogels		
15%	Pronase concentration (%w/v)	0.05	0.1	0.25	0.05	0.1	0.25
	Degradation time (min)*	110	85	65	185	175	110
	Collagenase concentration (%w/v)	-	-	0.5	-	-	0.5
	Degradation time (min)*	-	-	>120	-	-	>180

*Recovery percentage higher than 70%. Cell viability higher than 90%.

3. Standard cell recovery protocol

1. Completely remove the growth medium from each well containing hydrogels.
2. Add 100 µL of the prepared enzyme solution to each well.
3. Incubate the hydrogels at 37°C.
4. Monitor the degradation process at different time points using an inverted light microscope.

   Note: If the gel degradation is taking too long, and to prevent overexposure of the decapsulated cells to the enzyme solution, consider recovering the cells in suspension halfway through the process and then repeating the procedure starting from step 2.

5. Upon complete degradation of the cell-laden hydrogels, transfer the cell suspension from each well into microcentrifuge tubes.
6. Wash each well with medium and add the washings to the respective cell suspensions.
7. Centrifuge the cell suspensions at room temperature (RT) and 300 g for 5 minutes.

Note: Adjust the centrifuge conditions according to the sensitivity of the cells in use.

Keypoint notes:

1. Ensure all procedures are conducted under sterile conditions to prevent contamination.
2. The presented conditions are for reference only. Please, monitor the enzymatic degradation closely to determine the optimal time for cell recovery without minimal cell damage.
3. Adjust enzyme concentrations and incubation times, if necessary, based on initial observations.
4. Different proteolytic enzymes might be used. Collagenase IV and trypsin were previously tested but demonstrated inferior performance compared to pronase.

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or contact support@metatissue.com