PhotoCol®-RUT, Methacrylated Collagen Hydrogel Kit

3D CC Hydrogel

Cat. # CC322

pack size: 1 Kit

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Data Sheet

Background

3D cell culture, including bioprinting, allows for the creation of more physiological cell models by allowing cells to simultaneously interact with integrins on all cell surfaces, resulting in the activation of specific signaling pathways not activated in traditional 2D cell culture methods. Hydrogels are water swollen polymers that allow for the culture of cells in 3-dimensions and can have profound effects on cellular development, differentiation, migration, and function. New areas of tissue engineering such as 3D bioprinting, have utilized UV photocrosslinked methacrylated hydrogel biomaterials (PEGMA, GelMA, HAMA and ColMA etc.) to encapsulate cells to make printable bioinks.

The PhotoCol®-RUT, Methacrylated Collagen Hydrogel Kit is based upon purified type I bovine collagen methacrylate (ColMA), which when photocrosslinked provides a native-like 3D environment for cells. In addition to type I bovine collagen methacrylate, the kit includes the photoinitiator ruthenium/sodium persulfate and other reagents for users to easily fine tune their photocrosslinking experiments (i.e. altering hydrogel stiffness or gelling speeds). The methacrylated Type I collagen is produced from telo-peptide intact bovine collagen that has been modified by reacting with the protein's free amines, primarily the ε-amines groups of the lysine residues as well as the α-amines groups on the N-termini. > 20% of the total lysine residues of the collagen molecule have been methacrylated. The collagen is extracted from bovine hide and contains a high monomer content that was isolated from a closed herd and purified using controlled manufacturing processes.

Kit Components

The PhotoCol®-RUT, Methacrylated Collagen Hydrogel Kit (CC322) contains:

1) CC322-1 (Store at Room Temp): Neutralization Solution, 1 X 10 mL (CS226408), Ruthenium Photoinitiator, 1 X 100 mg (CS226407), Sodium Persulfate Photoinitiator, 1 X 500 mg (CS226406).

2) CC322-2 (Store at 2-8°C): Methacrylated Collagen, 1 X 100 mg (CS226410), 20 mM Acetic Acid, 1 X 50 mL (CS226409).

Note: Do not freeze reagents.

Quality Control

Sterility (USP modified): No Growth
Endotoxin LAL: ≤ 10.0 EU/ml
Gel Formation: ≤ 40 min
Kinetic Gel Test: ≤ 40 min
Purity (SDS Page Silver Stain): ≥ 99%
Degree of Methacrylation: ≥ 20%
pH: 3.0-4.0
Osmolality: ≤ 40 mOsm/Kg H20
Young's Modulus: Characteristic
Electrophoretic Pattern: Characteristic

References

1) Isaacson, A, Swioklo, S. & Connon, C. J. 3D bioprinting of a corneal stroma equivalent. Experimental Eye Research 173, 188–193 (2018).

2) Maloney, E, et al. Immersion Bioprinting of Tumor Organoids in Multi-Well Plates for Increasing Chemotherapy Screening Throughput. Micromachines 11, 208 (2020).

3) Lelièvre, S, et al. Cell Culture and Coculture for Oncological Research in Appropriate Microenvironments. Current Protocols Chem Biol. 2019 Jun;11(2):e65.

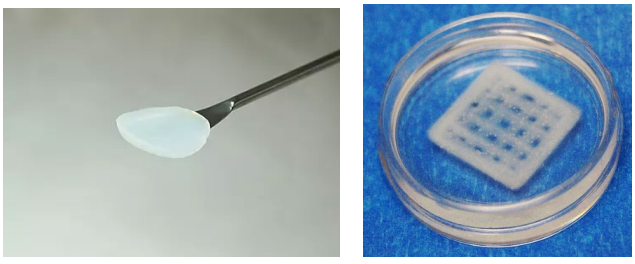

Figure 1. 3D printing of PhotoCol® Methacrylated Collagen Hydrogels can be used as native bioinks for tissue engineering bioprinting applications.

Rev.1.0/2020-04-24/CC322/NJA

Instructions for Use

Note: Employ aseptic practices to maintain the sterility of the product throughout the preparation and handling of the collagen and other solutions. It is recommended that the collagen and other working solutions be chilled and kept on ice during the preparation of the collagen. Vortexing is not recommended at any step.

1. Add volume of 20 mM acetic acid to the lyophilized methacrylated collagen to achieve desired concentration. Recommend concentration(s) range from 3 to 8 mg/ml.
2. Mix on a shaker table or rotator plate at 2-8°C until fully solubilized or overnight. Avoid formation of air bubbles as possible. *Note: The higher concentrations of collagen will take longer to solubilize.*
3. Determine the volume of the neutralization solution (NS) to mix with the collagen. To achieve a final pH of 7.0 to 7.4, follow the guidelines below.

Collagen Concentration	Collagen Weight	Neutralization Solution Volume
3 mg/mL	1.0 g	100 µL
4 mg/mL	1.0 g	114 µL
6 mg/mL	1.0 g	120 µL
8 mg/mL	1.0 g	128 µL

4. Transfer the required volume of the neutralization solution (NS) into a sterile vessel or tube and chill.
5. If photocrosslinking is desired, calculate the volume of each photoinitiator required by multiplying the total volume of collagen and neutralization solution by 0.02. If the resulting number is 100 ul, you will add 100 ul of ruthenium and 100 ul of sodium persulfate. Solubilize the required amount of Ruthenium (per step 6) at a concentration of 37.4 mg/ml in 1X PBS or cell culture media. Solubilize the required amount of sodium persulfate (per step 6) at a concentration of 119 mg/ml in 1X PBS or cell culture media.
6. Add the calculated volume of ruthenium photoinitiator to the volume of neutralization solution (NS) and mix thoroughly. Add the calculated volume of sodium persulfate photoinitiator to the volume of collagen solution and mix thoroughly.
7. Transfer the total volume of the chilled collagen/sodium persulfate into the chilled neutralization solution/ruthenium. Mix quickly and thoroughly by pipetting or rotating a vessel or tube. Do not vortex. *Note: Keep the collagen mixture chilled throughout this process. Check to ensure the pH is neutral. The high viscosity of this material can make it harder to mix.*
8. If desired, add dispersed chilled cells to the collagen mixture. Mix quickly and thoroughly by pipetting or rotating a vessel or tube. *Note: If air bubbles are a concern, allow to sit on ice until the bubbles come to the surface.*
9. Dispense the collagen mixture in the desired sterile plates or culture vessels.
10. Incubate at 37°C for > 30 minutes for gel formation.
11. If crosslinking is desired, place directly under a 400-450 nm light source. *Note: The consistency and fidelity of crosslinking is improved by plating gels on glass-bottom substrates with good optical properties that produce minimal light scattering.*