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Directions for Use
PhotoCol® - RUT

PURIFIED METHACRYLATED TYPE I COLLAGEN WITH RUTHENIUM KIT

Catalog Number **#5271-1KIT**

Product Description

Three dimensional (3D) gels allow for the study of the effects of the mechanical properties of the extracellular matrix (ECM), such as density and rigidity, on cell development, migration, and morphology. Unlike 2D systems, 3D environments allow cell extensions to simultaneously interact with integrins on all cell surfaces, resulting in the activation of specific signaling pathways. Gel stiffness or rigidity also affects cell migration differently in 3D versus 2D environments.

Furthermore, integrin-independent mechanical interactions resulting from the entanglement of matrix fibrils with cell extensions are possible in 3D systems, but not in 2D systems where the cells are attached to a flat surface.

Advanced BioMatrix offers PhotoCol®, a purified methacrylated Type I bovine collagen kit, which provides native-like 3D collagen gels with the unique attribute of being tunable when prepared at various concentrations and crosslinked with visible light.

The PhotoCol® kit consists of purified methacrylated Type I bovine collagen as the core component with other support reagents in the kit. Table 1 provides a list of the kit components.

Table 1:

Item	Catalog No.	Package Size
Collagen, Type I, methacrylated, lyophilized	5198-100MG	100 mg
Acetic Acid, 20 mM solution	5079-50ML	50 ml
Neutralization solution	5205-10ML	10 ml
Photoinitiator Ruthenium	5246-100MG	100 mg
Photoinitiator Sodium Persulfate	5247-500MG	500 mg

The methacrylated Type I collagen is produced from telo-peptide intact bovine collagen where the collagen has been modified by reacting the 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. The collagen starting material was isolated from a closed herd and purified using controlled manufacturing processes.

The 20 mM acetic acid solution is provided to solubilize the lyophilized methacrylated collagen at concentrations ranging from 3 to 8 mg/ml.

The neutralization solution consists of an alkaline 10X phosphate buffered saline (PBS) solution which provides physiological salts and neutral pH in the final mixture.

The photoinitiator solution consists of Ruthenium and Sodium Persulfate to be formulated in 1X PBS or cell culture media which allows visible light photocrosslinking of the collagen at 400-450 nm.

To sterilize, resuspend and filter each component **separately** through a 0.2 micron button filter.

Characterization and Testing

The formulated PhotoCol® has the following characteristics as shown in Table 2.

Table 2:

Test	Specifications
Purity by SDS PAGE electrophoresis	≥ 98%
Gel tube assay	≤ 10 minutes
Kinetic gel assay	≤ 10 minutes
Gel Stiffness	See graph 1 below
Differential Scanning Calorimetry (DSC) Thermal Analysis	Characteristic
Sterility	No growth
Endotoxin	≤ 10 Eu/ml

Storage/Stability:

The product ships on frozen gel packs. Upon receipt, store the collagen and acetic acid at 2-8°C and photoinitiator at room temperature. Do not freeze. Store the neutralization solution at room temperature.

The expiration date is printed on the product label and certificate of analysis for each specific lot as appropriate. The expiration date is applicable when product is handled and stored as directed. After solubilization of

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the collagen with acetic acid, the collagen solution is stable for 2 months when stored at 2 to 10°C.

Gel Stiffness:
The PhotoCol® kit is designed to provide collagen gels with varying gel stiffness based on collagen concentration and crosslinking. Light intensity, protein concentration, photoinitiator concentration, Photocrosslinking time, and other variables will affect polymerization performance.

Preparation Instructions

Note: Employ aseptic practices to maintain the sterility of the product throughout the preparation and handling of the collagen and other solutions.

Note: It is recommended that the collagen and other working solutions be chilled and kept on ice during the preparation of the collagen.

Note: Vortexing is not recommended at any step.

1. Add volume of 20 mM acetic acid (shown below) to the lyophilized methacrylated collagen to achieve desired concentration. Recommend concentration(s) range from 3 to 8 mg/ml.

Table 3:

Desired PhotoCol® Concentration	Volume of 20 mM Acetic Acid
3 mg/ml	33.3 ml
4 mg/ml	25.0 ml
6 mg/ml	16.7 ml
8 mg/ml	12.5 ml

2. Mix on a shaker table or rotator plate at 2-10°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 desired volume of collagen required.

4. 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 in Table 4 or Table 5.

Note: Dispensing by weight verses volume varies due 1) to the different viscosity of the different collagen concentrations and 2) sample hold up in the pipet tip.

Table 4:
Collagen to Neutralization Solution by Weight:

Solubilized Collagen Concentration	Weight of Collagen	Volume of NS
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

Table 5:
Collagen to Neutralization Solution by Volume:

Solubilized Collagen Concentration	Volume of Collagen	Volume of NS
3 mg/ml	1.0 ml	95 µl
4 mg/ml	1.0 ml	90 µl
6 mg/ml	1.0 ml	85 µl
8 mg/ml	1.0 ml	80 µl

5. Transfer the required volume of the neutralization solution (NS) into a sterile vessel or tube and briefly chill. Note – if the neutralization solution is chilled too long, the salts will come out of solution.

6. 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.

7. Solubilize the required amount of Ruthenium (per step 6) at a concentration of 37.4 mg/ml in 1X PBS or cell culture media.

8. Solubilize the required amount of sodium persulfate (per step 6) at a concentration of 119 mg/ml in 1X PBS or cell culture media.

9. Add the calculated volume of ruthenium photoinitiator to the volume of neutralization solution (NS) and mix thoroughly.

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10. Add the calculated volume of sodium persulfate photoinitiator to the volume of collagen solution and mix thoroughly.

11. 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.

Note: Check to ensure the pH is neutral. The high viscosity of this material can make it harder to mix.

12. 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.

13. Dispense the collagen mixture in the desired sterile plates or culture vessels.

14. To form gel, incubate at 37°C in humidified incubator for 30 minutes or until a firm gel is formed.

15. 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.

References

1. Gaudet, I. D., Characterization of Methacryated Type-I Collagen as a Dynamic Photoactive Hydrogel, Biointerphases, 2012 Dec; 7(1): 25.

2. Drzewiecki K. E., Methacrylation Induces Rapid, Temperature-Dependent, Reversible Self-Assembly of Type-I Collagen, Langmuir. 2014 Sep 23; 30(37): 11204–11211.