

## Directions for Use PhotoCol® - IRG

### PURIFIED METHACRYLATED TYPE I COLLAGEN WITH IRGACURE KIT Catalog Number #5201-1KIT

#### 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 UV 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 Irgacure 2959	5200-100MG	100 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  $\epsilon$ -amines groups of the lysine residues as well as the  $\alpha$ -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 Irgacure 2959 to be formulated in methanol (not included) which allows UV crosslinking of the collagen at 365 nm.

#### Characterization and Testing

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

Table 2:

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

#### Storage/Stability:

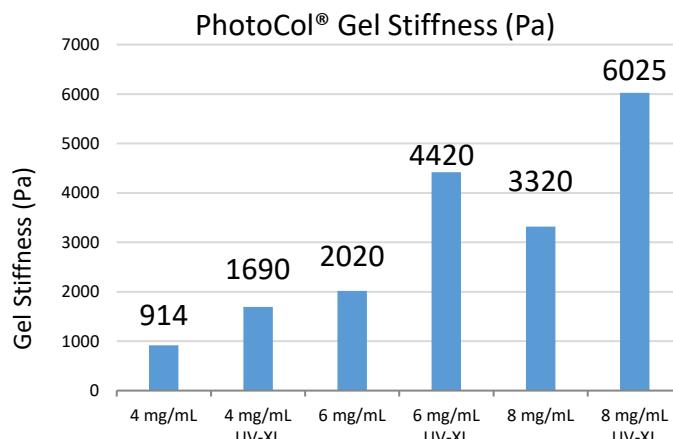
The product ships on frozen gel packs. Upon receipt, store the collagen, acetic acid and photoinitiator at 2 to 10°C. Do not freeze. Store the neutralization solution at room temperature.

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 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. Graph 1 shows typical gel stiffness results of PhotoCol® at varying concentrations with and without UV crosslinking.\*

Graph 1:



\*Rheology testing was done on a Bohlin CVO-100 rheometer. Crosslinked collagen was exposed to 365 nm UV light for 5 minutes.

## 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 gets too cold, it will come out of solution).
6. If crosslinking is desired, first add 1 mL of neat methanol to the amber vial containing 100 mg of Irgacure, and vortex. (Irgacure only has a 2 week shelf life upon solubilizing. If you need the

7. Irradiate to last longer, remove required amount and solubilize (10% solution).
8. Calculate the volume of the photoinitiator required by multiplying the total volume required (collagen and neutralization solution) by 0.01.
9. Add the calculated volume of chilled photoinitiator to the volume of chilled neutralization solution (NS) and mix thoroughly.
10. Transfer the total volume of the chilled collagen into the chilled neutralization solution (NS)/photoinitiator. Mix quickly and thoroughly by pipetting or rotating a vessel or tube.

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.

11. 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.

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

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

14. If crosslinking is desired, place directly under a 365 nm UV light crosslinking source.

Tunability of PhotoCol® product exposed to UV for 45, 90 and 600 seconds results respectively in a 22, 53, and 75% increase in gel stiffness while maintaining a stable gel stiffness over time.

Longer exposure allows more crosslinking, though each cell type withstands different degrees of UV light and the free radicals (generated by the photoinitiator) that mediate crosslinking.

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 Methacrylated 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.