Directions for Use
LifeInk® 100

PURIFIED METHACRYLATED TYPE I COLLAGEN KIT FOR CROSSLINKABLE BIOINKS

Catalog Number **#5204-1EA**

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 LifeInk® 100, a purified methacrylated Type I bovine collagen kit, which provides native-like 3D collagen gels with the unique attributes to be prepared at various concentrations and crosslinked to provide various gel stiffness.

The LifeInk® 100 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. The entire kit is included to allow the neutralization of LifeInk® 100 prior to printing, though due to the quick gelation properties of this material, we have included our recommended protocol which does not require the neutralization solution.

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	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 ε-amines groups of the lysine residues as well as the α-amines groups on the *N*-termini. Approximately 40% 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 pH in the final mixture.

The photoinitiator solution consists of Irgacure 2959 which needs to be formulated in methanol (not included) allowing for UV crosslinking of the collagen at 365 nm.

Characterization and Testing

The formulated LifeInk® 100 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, acetic acid and photoinitiator at 2 to 10°C. Do not freeze. Store the neutralization solution at room temperature.

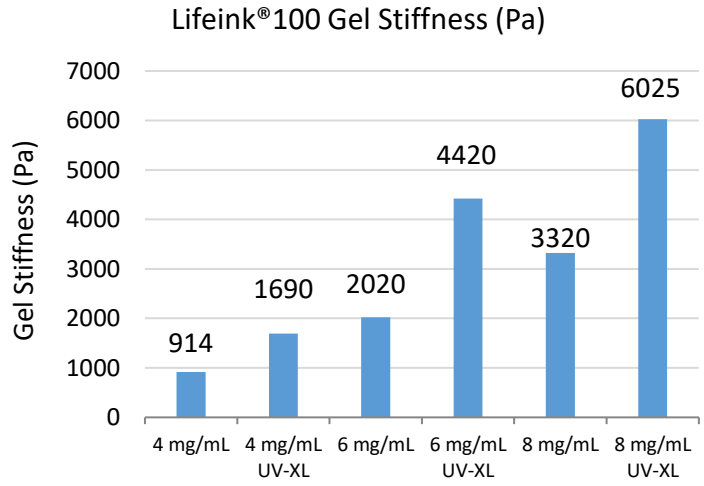
The expiration dates are printed on the product label and

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 LifeInk® 100 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 LifeInk® 100 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 – Acidic Printing

We recommend the following preparation procedures for 3D printing LifeInk® 100. Due to the fast gelling nature of telocollagen (the base material pre-methacrylation), we recommend employing a dual extruder system and printing the collagen and cells separate rather than printing the two in one syringe.

We recommend printing LifeInk® 100 as an acid, into a pH neutral/isotonic buffer (such as FRESH gelatin slurry solubilized in 1X PBS).

We are conducting further testing of printing neutralized/cell encapsulated LifeInk® 100 in cold temperatures.

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 collagen 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 for 3D Bioprinting is 8-10 mg/ml for higher viscosity and print resolution.

Table 3:

Desired LifeInk® 100 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
10 mg/ml	10 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. If crosslinking is desired, first add 1 mL of neat methanol to the small amber vial of photoinitiator containing 100 mg of Irgacure, and vortex. (Note – Irgacure in solution has a shelf life of 2 weeks. Only dissolve required amount of Irgacure powder in a 10% solution)

4. Calculate the volume of the photoinitiator required by multiplying the total volume of collagen required by 0.02.

5. Add the calculated volume of chilled photoinitiator to the collagen solution and mix thoroughly.

   Note: Keep the collagen mixture chilled throughout

this process.

Note: If air bubbles are a concern, allow to sit on ice until the bubbles come to the surface.

6. Dispense the collagen mixture into the desired printing mechanism (i.e.10cc syringe).

   Note: To allow gelation of the collagen, neutral pH and physiological salts are required. Ensure support media contains 1X salts and is neutral pH.

7. Print LifeInk® 100 acidic solution into the support media. Gelation of collagen should occur within 2 minutes. Allow collagen to gel prior to UV crosslinking for best results.

8. If crosslinking is desired, place directly under a 365 nm UV light crosslinking source.
9. To incorporate cells, we recommend two options:
   a. Employ a dual extruder system. Cells can be suspended in our LifeInk® 200 high concentration collagen #5202-1EA.

   b. Post UV-Crosslinking, seed cells on the printed construct.

Tunability of LifeInk® 100 product exposed to UV for 45, 90 and 600 seconds results respectively in a 22, 53, and 75% increase in gel stiffness.

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

Preparation Instructions – Neutralized Printing

Note – due to the quick gelling nature of telocollagen, the material must be kept at 4C to slow down gelation. We do not recommend storing the neutralized collagen for any period of time, even at 4C.

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	12.6 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 chill.

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 Irgacure to last longer, remove required amount and solubilize (10% solution).

7. Calculate the volume of the photoinitiator required by multiplying the total volume required (collagen and neutralization solution) by 0.01.

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

9. 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.

10. 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.

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

12. Incubate at 37°C for $\geq$ 30 minutes for gel formation.

13. 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 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.