

## Directions for Use

### Lifeink® 200

COLLAGEN BIOINK, 35 MG/ML  
Catalog Number #5278-5ML

#### 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.

A bioink that resembles life should include the following traits: high print resolution, shear-thinning and recovery, strong mechanical strength, superior cytocompatibility, allows for cellular remodeling, and is biomimetic. Lifeink® 200 fulfills those requirements.

Advanced BioMatrix offers Lifeink® 200, a bioink that is a highly concentrated type I collagen. Lifeink® 200 is pH neutral collagen with physiological salt concentration. Lifeink® 200 needs to be printed at 2-8°C into LifeSupport® (FRESH Printing) for optimal results.

The collagen is extracted from bovine hide and contains a high monomer content. The collagen starting material and purified using controlled manufacturing processes.

#### Characterization and Testing

The formulated Lifeink® 200 bioink has the following characteristics as shown in Table 2.

Table 2:

Test	Specifications
Purity by SDS PAGE electrophoresis	≥ 98%
Gel Stiffness	See graph 1 below
Sterility	No growth
Endotoxin	≤ 10 Eu/ml
pH	Neutral

#### Storage/Stability:

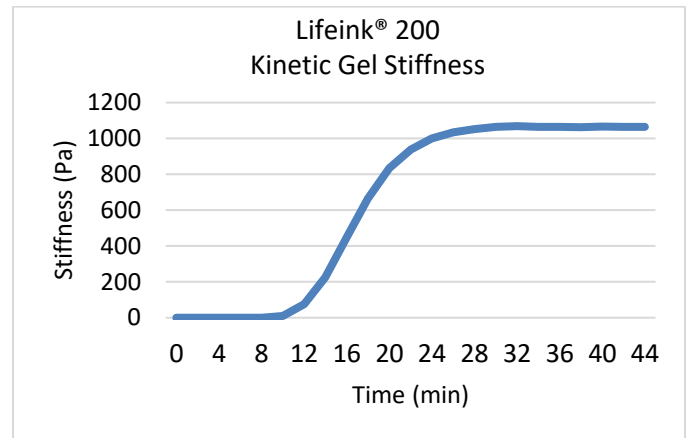
The product ships on frozen gel packs. Upon receipt, store the collagen at 2 to 10°C. Do not freeze.

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.

#### Gel Stiffness:

The Lifeink® 200 kit is designed to provide 3D printed collagen gels. Graph 1 shows typical gel stiffness results of Lifeink® 200 at 37°C.

Graph 1:



\*Rheology testing was done on an Elastosens® rheometer at 37°C.

#### Preparation Instructions for Lifeink® 200

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 during the preparation of the collagen.

Note: Vortexing of collagen is not recommended at any step.

Note: Ensure that NO bubbles enter the system. Bubbles in the system while mixing will turn your ink into a foam-like material.

Note: We recommend printing Lifeink® 200 at 2-8°C into a gelatin slurry support (FRESH method) for best results.

#### Cell Addition to Bioink:

Note: Keep collagen chilled throughout these processes

1. Add the concentrated cell suspension in cell culture media to a sterile syringe.

[Video for step 1](#)

Note: We recommend a final bioink cell concentration of  $5 \times 10^6$ /mL or greater.

Note: For best results, add 2 mL of cell suspension per 5 mL of collagen Bioink. Use a similar ratio for smaller volumes.

2. Place sterile coupler on the end of the syringe with the cell suspension.
3. Slowly push plunger in until media forms a slight external meniscus above the end of the coupler on the syringe.
4. Remove cap from the syringe with collagen and slowly push plunger in until collagen forms a slight external meniscus above the end of the syringe.
5. Couple the syringe with cells to the syringe with collagen. **(Ensure that there are no air bubbles in the system. The “external meniscus” on both syringes helps ensure that there are no air bubbles introduced).**
6. Slowly push plungers back and forth ~40 times to ensure thorough mixing. End with all of the material in the syringe to be used for printing.

[Video for steps 2-6](#)

7. The cell-laden bioink is now ready for positive displacement printers.

Note: For pneumatic printers, transfer the collagen into an appropriate syringe using the

coupler. The new syringe should have the seal inserted, but the plunger removed. Centrifuge the syringe at 2000 RPM for 1 minute after transferring the collagen to remove any air bubbles. DO NOT CENTRIFUGE the syringe if you have added cells to the syringe.

[Watch this video for help](#)

#### General Printing Notes:

1. To use a smaller volume of collagen, simply transfer the desired amount of collagen to another syringe, using the provided sterile coupler. To remove the air from the new syringe, you can do either of the following:
  - a. Centrifuge the syringe (capped) with the cap pointing up to cause the air to accumulate at the cap. Evacuate the air.
  - b. Centrifuge the syringe (capped) with the cap pointing down, and then use a hemostat to squeeze the syringe while pushing the plunger to allow the air to escape.

[Removing air with a hemostat video](#)

2. Complete your print within 1 hour of cell addition to syringe/bioink for best cell viability results.
3. When printing with FRESH gelatin slurry, allow the final printed structure to incubate at 37°C for 30 to 60 minutes and then replace the gelatin with media.
4. Avoid bubbles.