

Directions for Use

Lifeink® 240

ACIDIC COLLAGEN BIOINK, 35 MG/ML
Catalog Number #5267-5ML

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, strong mechanical strength, superior cytocompatibility, allows for cellular remodeling, and is biomimetic. Lifeink® 240 fulfills those requirements.

Lifeink® 240 is a Type I collagen bioink at a concentration of 35 mg/ml for extrusion-based 3D bioprinting. The product is acidified collagen intended to be extrusion printed employing FRESH bioprinting (see LifeSupport® Catalog No. 5244-8GM). Lifeink® 240 produces printed structures with a high print resolution and good mechanical strength. The product is formulated in an acidic saline buffer solution. Once the collagen is printed into LifeSupport®, the pH and salts concentration of the printed structure becomes physiological. Cells can then be printed alongside the extruded collagen, or cells can be seeded onto the printed structure post-print, allowing for cell adherence and cellular remodeling of the 3D bioprinted structure.

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

Characterization and Testing

The formulated Lifeink® 240 bioink has the following characteristics as shown in Table 1.

Table 1:

Test	Specifications
Appearance	Transparent Clear Solution
Sterility	No growth
Endotoxin LAL (EU/ml)	≤ 10
Collagen Concentration (mg/ml)	30 to 40
Purity by SDS PAGE Electrophoresis ⁽¹⁾	Characteristic
SDS PAGE Silver Staining ⁽¹⁾	≥99%
Rheology	Characteristic
Bioprintability	Passes
Continuous extrusion Flow (30-gauge needle or larger – no needle clogging)	Pass
pH	3.0 to 5.0
Osmolality (mOsmo H ₂ O/kg)	450 to 700

(1) Testing performed on raw material.

Storage/Stability:

The Lifeink® 240 bioink ships on frozen gel packs. Upon receipt, store the collagen at 2 to 10°C. Do not freeze.

Shelf life of Lifeink® 240 bioink is under evaluation and has a minimum shelf life of 12 months from date of receipt.

Preparation Instructions for Lifeink® 240

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

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



Note: Cells should not be added directly to the Lifeink® 240 bioink since it is an acidic formulation.

Addition to other components to Lifeink® 240 Bioink:

Note: Components other than cells can be added to the Lifeink® 240 bioink before bioprinting as long as these components are compatible with acidic pH conditions.

1. To mix in additives to the bioink, add the additives to a secondary syringe as demonstrated in the video below:

[Video for step 1](#)

Note: The video shows cells being added to the bioink. Other materials can be added to the Lifeink® 240 bioink in lieu of cells. Cells should NOT be added directly to this bioink. For cellular bioprinting, use Lifeink® 200 #5278-5ML.

Note: For best results, add no more than 5 mL of other components 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 bioink additives.
3. Slowly push plunger in until solution 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 additives 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 bioink with other components is now ready for extrusion 3D bioprinters.

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.

[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. 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.
3. Avoid bubbles.

For more directions on FRESH printing, please visit our LifeSupport® Directions for Use.