ADVANCED BIOMATRIX
A BICO COMPANY

Lifeink® Collagen Bioink Starter Kit
Directions for Use

Note: Prior to using the Lifeink® bioink start kit, we strongly recommend printing with other materials (ie. Cellink Start or Pluronic F-127) to get accustomed to the printer, print settings, material handling and other aspects of bioprinting.

Kit Description

The Lifeink® collagen bioink starter kit is designed to enable immediate printing of pure collagen. The kit contains the following items:

Item	Quantity
Lifeink® 200	1 x 3 mL
Lifeink® 240	1 x 3 mL
LifeSupport®	2 x 2 grams
35 mm dishes	4 dishes
25-gauge needles	50 needles (0.5”)

Product Descriptions

Lifeink® 200 is a 35 mg/ml, pH neutral, isotonic collagen bioink. The consistency is “paste-like.” Cells can be directly added to this bioink and printed through small diameter needles due to its shear thinning ability. For using the Lifeink® 200 as found in the starter kit, we recommend first doing *acellular* printing to better understand the printing parameters and mechanical properties of the collagen.

Lifeink® 240 is a 35 mg/ml, acidic, collagen bioink. While the 200 (above) is paste-like, Lifeink® 240 is a viscous, concentrated solution. It requires higher pressure to print but can go through even smaller diameter needles (with increased pressures).

The FRESH printing method is strongly recommended with Lifeink® collagen bioinks. FRESH printing is performed by extruding bioinks within a specially formulated support bath. This bath is designed to promote high resolution printing of soft biomaterials, such as collagen, while preventing constructs from collapsing and deforming.

A visual representation of FRESH printing can be seen here.

LifeSupport® tubes contain 2 grams of gelatin microparticles for creating FRESH support baths. Each tube typically rehydrates to 20 mL of slurry, or approximately enough to fill 2 of the 35mm dishes.

Starting Print Parameter Recommendations

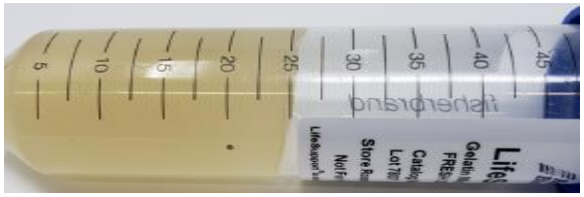
	Lifeink® 200	**Lifeink® 240**
Needle	0.5” 25-gauge	0.5” 25-gauge
Air pressure	~70 kPa	~200 kPa
Layer Height	260 um	260 um
Print Temp	2-8C recommended	2-25C
Print Speed	14 mm/s	7 mm/s

The above notes are print recommendations for the bioinks and needles as supplied in the starter kit. To order more Lifeink®, expand your printing capabilities (such as printing with a 30-gauge needle, or adding cells), and see a more comprehensive directions for use, visit our website and find Lifeink® products under the “Bioprinting” tab.

LifeSupport® Preparation

Note: *Keep cold where possible.* Warming up the gelatin will melt the microparticles and disable FRESH printing.

1. Aliquot 1 gram of LifeSupport® powder into a separate 50 mL conical tube.
2. Add 40 mL of cold (4°C) 1X PBS or pH neutral cell culture media to the 1 gram of powder.
3. Vortex and shake vigorously for 1 minute to ensure resuspension.
4. Let stand at 4°C for 15 minutes to allow full rehydration.
5. Centrifuge at 2000 x g for 5 minutes. The gelatin should appear compact at the bottom of the tube.
6. Pour out the separated liquid.
7. Re-cap the tube and shake the tube to dislodge the compact gelatin pellet.
8. Re-centrifuge at 2000 x g for 5 minutes a second time.
9. Gently pour off remaining liquid. At this point, LifeSupport® should stay in place when the tube is tilted sideways.



10. Scoop out the LifeSupport® into the 35mm dish and spread until even.
11. Keep cold and use within 2 hours of resuspending and compacting the LifeSupport®.

Lifeink® Printing

Note: The Lifeink® 200 and 240 formats/syringes as found in the Bioink Starter Kit were designed for "plug-and-play" collagen bioprinting. For customization of the bioinks (ie. adding cells, proteins, etc…), please order the larger Lifeink® syringes with plungers.

1. Ensure printing parameters are established per the recommendations on page 1.
   Note: we recommend starting with a simple square construct 20mm x 20mm x 3mm with 15% infill.
2. Remove Lifeink® cartridge from the refrigerator and attach the 25-gauge needle. Insert into BioX printer. Attach air pressure to the cartridge.
3. Perform a "test flow" to ensure collagen flow through the needle.
4. Lifeink® 200 prints best when kept cold (increased viscosity = more consistency and better resolution). If you do not have the BioX temperature-controlled printhead, keep the collagen in the refrigerator or on ice until right before printing. Lifeink® 240 prints well from 2-25°C. Set the printbed temperature to 10°C to keep the LifeSupport chilled.
5. After all the parameters are set (see page 1), calibrate the needle tip to define the origin of the print. The needle tip should be fully submerged in the LifeSupport® slurry, about 1mm from the bottom of the dish, and in the middle (x,y) to avoid crashing into the walls.
6. Print.

Post-Printing

1. After printing, incubate the 35mm dish at 37°C. The Lifeink® 200 construct will be immediately visible. The Lifeink® 240 will gradually appear over time as it polymerizes. After 45 minutes, the gelatin will be fully melted, and your collagen construct should be floating.
2. Using a pipettor, carefully aspirate out 2 mL of melted LifeSupport, and add 2 mL of warm cell culture media. Repeat until most of the melted gelatin slurry has been replaced by media.