

Directions for Use

LifeSupport™

SUPPORT SLURRY FOR FRESH 3D BIOPRINTING
Catalog Number #5244-8GM

Product Description

LifeSupport™ is the core component and industry standard for FRESH bioprinting. FRESH stands for Freeform Reversible Embedding of Suspended Hydrogels. Each LifeSupport™ printing kit comes with 4 units of 2 grams of sterile, LifeSupport™ powder. One gram of powder hydrates to approximately 10 mL of FRESH support material.

FRESH printing implements a specially formulated support bath that prevents constructs from collapsing and deforming while printing. A wide array of polymer chemistries is supported with FRESH printing by the incorporation of ions, enzymes, pH buffers and more into your support bath for your given bioink. Please reference this publication for a full description:

<http://advances.sciencemag.org/content/1/9/e1500758.full>

FRESH bioprinting with LifeSupport™ allows for complex 3D bioprinting, without the need for sacrificial inks (Pluronic F-127, polycaprolactone, gelatin) or ink modifiers that increase ink mechanical stability (gelatin methacrylate, cellulose, alginate).

LifeSupport™ can be resuspended in a variety of media to support a wide range of biomaterials. Only slight adjustments to centrifugation speed need to be made to comply with different media (listed below).

Bioinks that can be printed include collagen, alginate, fibrin, decellularized extracellular matrix, methacrylated hyaluronic acid, and more. The specific bioinks that can be printed will also depend on the hardware capabilities of the 3D bioprinter.

LifeSupport™ suspended in cell media is optimal for bioprinting as cells immediately receive media upon being printed in LifeSupport™.

Characterization and Testing

LifeSupport™ has the following characteristics as shown in Table 1.

Table 1:

Test	Specifications
Average Particle Size Diameter	15-50 micron
Sterility Testing	No growth
Weight	8 grams of powder (4 X 2 grams)
Rheology	Bingham plastic
Shelf Life	Under evaluation

Storage/Stability:

The product ships and is stored at room temperature.

Limit exposure to air, as the material is highly hygroscopic.

Rehydrated LifeSupport™ is highly temperature sensitive, and will begin melting above 32°C.

Once hydrated with cold media, LifeSupport™ should be used within 12 hours.

Preparation Instructions for LifeSupport™

1. Transfer the required weight of LifeSupport™ powder into a 50 mL sterile conical centrifuge tube (see Table 2 for recommended weights). Do not add more than 2.1 grams of LifeSupport™ per tube, as this will result in an unequal compaction gradient of slurry post centrifugation. Note – to maintain sterility, all steps should be performed under sterile conditions using sterile plasticware and reagents.

2. Weigh out required powder into a 50 mL centrifuge tube, and then add recommended volume of **COLD** media as found in Table 2. Note – these volumes are to *fill* the printing dish, which is not needed for all prints. For example, printing a flat scaffold may only require the dish to be partially filled.

Table 2:

Printing Dish	Powder Weight	Media Volume
35 mm dish	1.0 gram	17 mL
6-well plate	2.10 grams	35 mL
12-well plate	0.86 grams	15 mL
24-well plate	0.43 grams	7 mL

LifeSupport™ can be customized to fit your specific bioink.

See the Table 3 below for help on choosing the right suspension media to take advantage of the unique benefits of FRESH bioprinting.

Table 3:

Bioink	Suspension Media	Centrifugation
Neutralized Collagen	Cell Culture Media or 1X PBS	2000G*; 5 min
Alginate	0.1 wt% CaCl ₂	2000G*; 5 min
Fibrinogen	Media with Thrombin	2500G*; 5 min
Matrigel	Cell Culture Media or 1X PBS	2000G*; 5 min
Acidic Collagen	50 mM HEPES buffer (pH 7.4)	2000G*; 5 min
Decellularized ECM	Cell Culture Media or 1X PBS	2000G*; 5 min

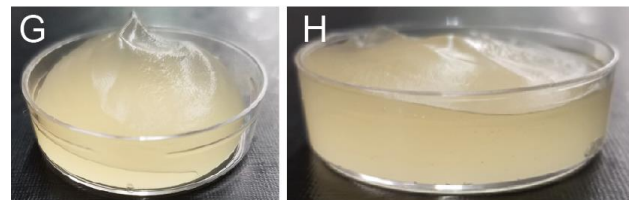
*Note: centrifugation speed and time may vary slightly depending on the suspension media. Serum-based growth media requires higher centrifugation speed (ie. 2500-3000G). 1X PBS is the easiest and most consistent to use.

3. Vortex for 1 minute, ensuring that all the powder is fully resuspended and then allow to sit for 10 minutes while the powder fully rehydrates.

4. Centrifuge the support material according to table 3.

5. Support material should be compacted at the bottom of the tube (see right). **Pour off the supernatant.**

6. Aseptically scoop out the compacted LifeSupport™ slurry into the desired printing dish using a sterile spatula or other device (see G and H). Avoid introducing large air bubbles in the slurry. The slurry should not move if the dish is held sideways.

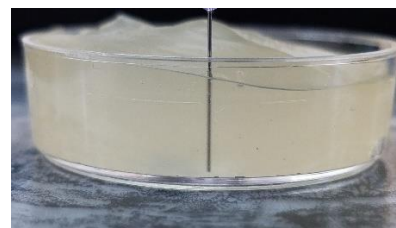


You are ready for FRESH bioprinting.

Printing Recommendations:

1. Place LifeSupport™ support slurry in your 3D bioprinter.

2. Calibrate (zero) your needle near the bottom of the dish (z), and near the middle (x,y). See below.



Note: Unlike typical printing, the needle does not have to be touching or even close to the bottom of the dish. The LifeSupport™ support slurry will trap your print in-place, no matter where you start. Ensure that the print container is large enough to avoid the needle hitting into the walls during printing.



3. Bioprinting can now be initiated. Ensure the container will not shift on the print bed.

Note – It is possible to print multiple constructs in the same container in order to maximize the number of prints per LifeSupport™ kit.

Post Print Recommendations:

1. After printing, incubate the printed construct at 37°C for at least 30 minutes to allow the LifeSupport™ slurry to melt and release the bioprinted construct.

2. Cellularized bioinks printed into cell media-based support material can be directly placed in a cell incubator for 24 hours before needing to exchange media (ensuring proper levels of CO₂).

3. After 30 minutes of post-print incubation, the LifeSupport™ slurry will be fully melted and your printed structure will be released. Large volumes may require longer times to fully melt.

4. Transfer of bioprinted construct:

4a. Carefully transfer bioprinted constructs into desired cell culture medium.

4b. As an alternative, melted LifeSupport™ slurry can be serially replaced with pre-warmed cell culture medium, to avoid handling the printed construct. For example, if you printed into a 6-well plate, this can be done by carefully aspirating out 2 mL of melted LifeSupport™ and adding 2 mL of pre-warmed cell culture medium. Repeat this process until all of the melted LifeSupport™ slurry has been replaced by medium.

5. Continue standard media changes in accordance with cell culture protocol.

LifeSupport™ is manufactured by FluidForm under license from Carnegie Mellon University Patent No. 10,150,258.