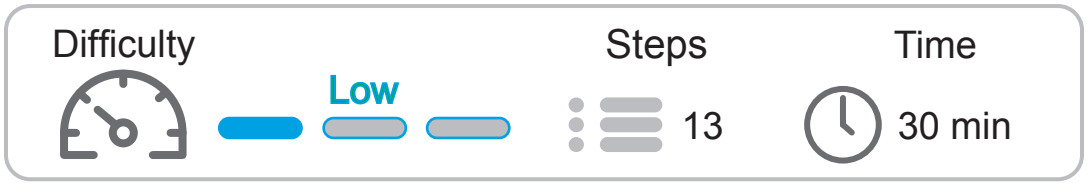
FLUIDFORM™
THE REGENERATIVE MEDICINE COMPANY

LIFESUPPORT™ DIRECTIONS FOR USE

SUPPORT BATH FOR FRESH™ 3D BIOPRINTING



PRODUCT DESCRIPTION

Each LifeSupport™ printing kit comes with 5 individual 2 g units of sterile, dried, LifeSupport™ powder which is composed of gelatin microparticles of defined size and shape. Each unit rehydrates to approximately 20 mL of LifeSupport™ support bath.

FRESH 3D bioprinting is performed by extruding bioinks and other materials within the hydrated, compacted LifeSupport™ bath, which is specially formulated to prevent constructs from collapsing and deforming while printing. A wide range of polymer crosslinking chemistries and gelation mechanisms can be supported within LifeSupport™ by the incorporation of ions, enzymes, pH buffers and more into the support bath during the rehydration process.

LifeSupport™ allows for FRESH 3D bioprinting of soft hydrogel bioinks in complex geometries without the need for sacrificial support inks (e.g., Pluronic® F-127, polycaprolactone, gelatin) or ink modifiers to increase mechanical stability (e.g., gelatin methacrylate, cellulose, alginate).

LifeSupport™ can be rehydrated in a range of buffers and cell culture media to support multiple cell types and specific bioinks. LifeSupport™ can also be rehydrated to support the cross-linking and/or gelation of multiple types of bioinks within the same container of support bath. Bioinks that can be printed include collagen, alginate, fibrin, decellularized extracellular matrix, methacrylated gelatin, methacrylated hyaluronic acid, and more. The specific bioinks that can be printed will also depend on the hardware capabilities of the 3D bioprinter that you are using.

Please refer to our website at www.fluidform3D.com for an up-to-date list of publications and examples of specific applications using FRESH 3D bioprinting.

If this is your first time using LifeSupport™, we strongly recommend taking advantage of the free FRESH Technical Assistance Program to smoothly get started. Contact us for more information regarding this program at info@fluidform3d.com.

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CHARACTERIZATION AND TESTING

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

Table 1: LifeSupport properties.

Test	Specifications
Weight	2 g per unit (dry)
Shelf Life	1 year (dry)
Sterility	Sterile
Average Particle Size	30 (+/-) 10 µm (rehydrated)
Rheology	Bingham plastic (rehydrated)

STORAGE/STABILITY:

The product ships and is stored at room temperature.

Limit exposure to air, as the dry LifeSupport™ is highly hygroscopic.

Rehydrated LifeSupport™ is highly temperature sensitive, and will begin melting above 32°C. Holding a container tightly can warm the material significantly. We recommend that preparation, handling, and printing be performed in a temperature controlled room at 23°C or less.

Rehydrated LifeSupport™ can be stored in the non-compacted state (i.e., prior to centrifugation) for 7 days under refrigeration to avoid degradation.

Once compacted, LifeSupport™ should be used **within 12 hours** and the temperature should not exceed 23°C during handling or printing.

PREPARATION INSTRUCTIONS FOR LIFESUPPORT™

Each unit of LifeSupport™ contains approximately 2 g of dry powder. Each 2 g unit of powder rehydrates to approximately 20 mL of the final support bath. The volume of support bath can be scaled accordingly to the amount of powder used.

Ask your bioprinter manufacturer if they have FRESH-specific print settings to ensure the highest quality results!

Table 2: Ink-specific media and centrifugation speeds.

<table>
<tr><th>Bioink</th><th>Suspension Media</th><th>Centrifugation</th></tr>
<tr><td>Acidified Collagen</td><td rowspan="3">Any buffered solution at a pH of 7 to 8 (including Cell Culture Media, 1X PBS, and 50 mM HEPES)</td><td rowspan="8">1st Centrifugation 2000 X g; 5 min

2nd Centrifugation* 2000 X g; 5 min</td></tr>
<tr><td>Lifeink 240</td></tr>
<tr><td>Decellularized ECM</td></tr>
<tr><td>Alginate</td><td>0.1 wt% $CaCl_2$</td></tr>
<tr><td>Fibrinogen</td><td>Any solution with Thrombin</td></tr>
<tr><td>Neutral or buffered collagen</td><td rowspan="3">Cell Culture Media or 1X PBS</td></tr>
<tr><td>Basement Membrane</td></tr>
<tr><td>Ink with Cells</td></tr>
</table>

* = centrifugation speed and time may vary slightly depending on your suspension media. For serum-based growth media, centrifuge at a higher speed (ie. 2000 X g, 5 min) until compacted LifeSupport™ bath stays in place when the tube is slowly turned on its side (J).

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DIRECTIONS

1. For best preparation results, we strongly recommend splitting the 2 g unit of LifeSupport™ into 1 g aliquots.

   Add 40 mL of cold (4°C) suspension media to a 1 g aliquot of LifeSupport™ (A). See Table 2 for suspension media recommendations for different

A

<u>NOTE</u> To maintain sterility, all steps should be performed under sterile conditions using sterile plasticware and reagents.

<u>NOTE</u> To prevent premature melting of LifeSupport™, all suspension media should be refrigerated prior to use.

2. Vortex (B) and shake vigorously for 1 min to ensure all powder is fully resuspended (C) and not stuck to tube walls / tip (D).
3. Let stand for 15 minutes at 4°C to allow LifeSupport™ to fully rehydrate.

B

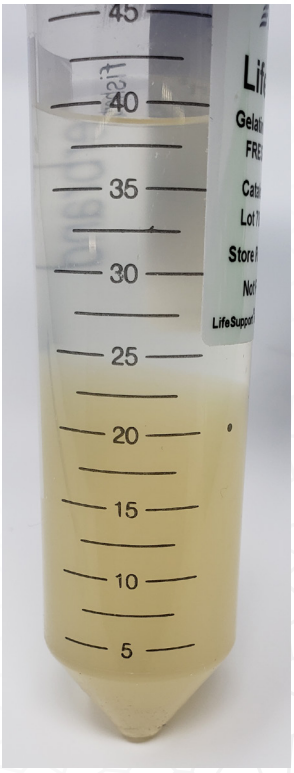
C

D

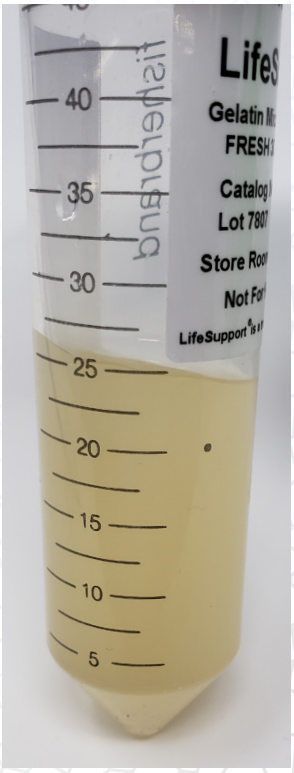
<u>OPTIONAL</u> Degas the support bath in a vacuum chamber for 30 min (E) to remove dissolved gases and prevent the formation of bubbles during printing.

E

4. Remove LifeSupport™ from 4°C, shake LifeSupport™ for 10 seconds. Centrifuge the rehydrated LifeSupport™ in a properly balanced centrifuge to compact it. Refer to Table 2 "1st Centrifugation" for recommended centrifuge settings.
5. The LifeSupport™ should now be compacted at the bottom of the centrifuge tube (F). Gently pour off or aspirate the liquid supernatant to leave only the compacted LifeSupport™ in the bottom of the centrifuge tube (G).



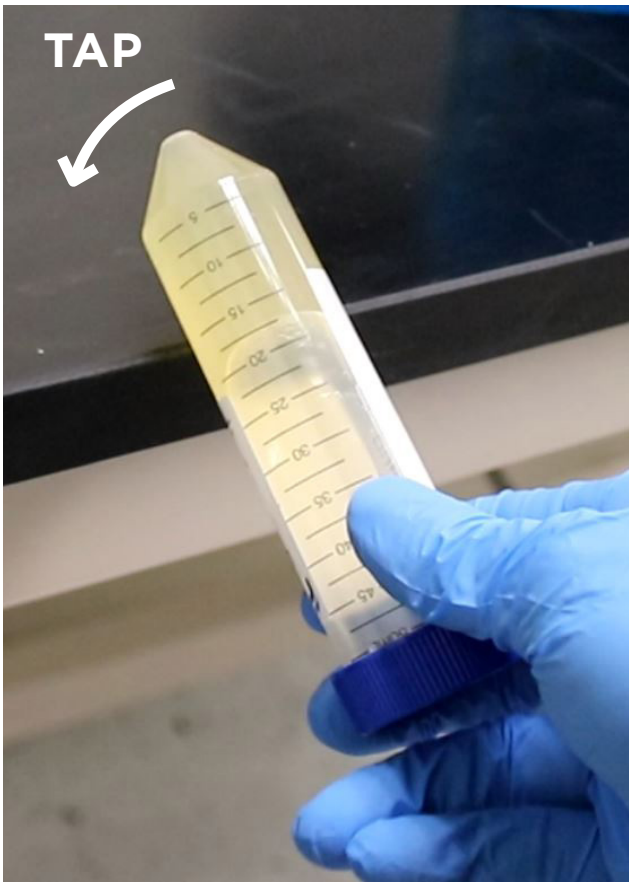
F



G

6. Cap the tube containing the compacted LifeSupport™.

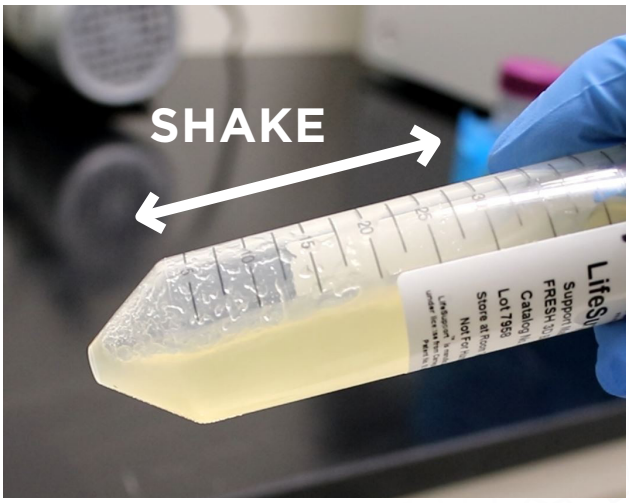
7. Grab the tube by the cap, hold it horizontally, and gently tap body of the tube against the edge of a table 15 times (H).



H

***NOTE** This is to dislodge the compacted LifeSupport™, allowing it to flow down towards the cap during tapping.*

8. Shake the tube containing dislodged LifeSupport™ vigorously for 10 seconds. Shake along the length of the tube (I).



I

***NOTE** User should feel LifeSupport™ moving and hitting the cap and inner surfaces of the tube during shaking.*

***NOTE** Hold tube by the cap during shaking and avoid handling the body of the tube.*

9. Centrifuge well-shaken LifeSupport™ in a properly balanced centrifuge to compact it. See Table 2, "2nd Centrifugation", for recommended centrifuge settings.

***NOTE** Use temperature-controlled centrifuge if possible. If this is not available, carefully monitor LifeSupport™ behavior after centrifugation. If your centrifuge warms up significantly during the centrifugation cycle, it may affect the performance of LifeSupport™. It is advisable to ensure LifeSupport™ is refrigerated enough to keep it cool during centrifugation.*

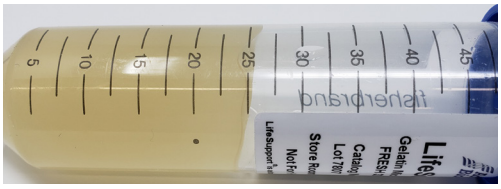
***NOTE** To create an accurate counter-balance for the centrifuge, measure the mass of the tube containing LifeSupport™ on an analytical balance.*

10. The LifeSupport™ should now be compacted at the bottom of the centrifuge tube. Gently pour off or aspirate any remaining liquid supernatant to leave only the compacted LifeSupport™ in the bottom of the centrifuge tube. At this point, LifeSupport™ should have the rheological behavior of a Bingham Plastic.

    LifeSupport™ has been prepared properly if the LifeSupport™ stays in place when the tube is slowly turned on its side (J). A small amount of flow is acceptable.

    <u>WARNING</u> If the LifeSupport™ flows easily in the tube (K), stop, resuspend in cold media, and repeat steps 4-10. In this case it may be necessary to increase the "2nd Centrifugation" speed in step 9 in 200 X g increments until LifeSupport™ is properly compacted.

J



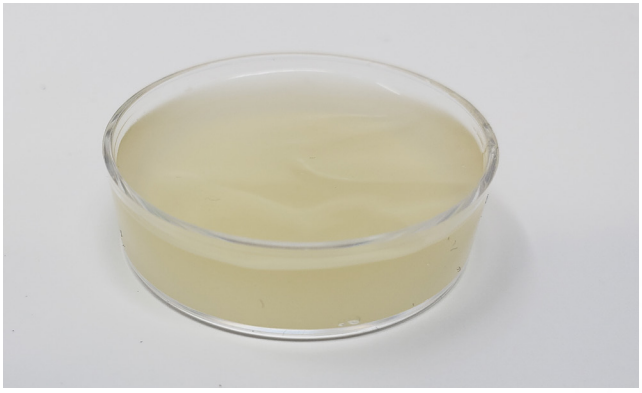
K

11. Aseptically scoop out or tap the compacted LifeSupport™ into the desired printing container (L) using a sterile spatula or other device.

L

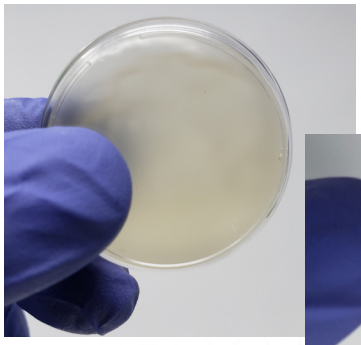
12. Tap the container against a surface to settle the bath in the container (M).

M

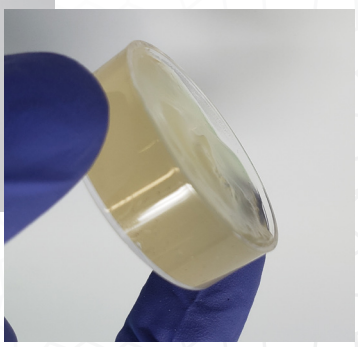


13. The bath should be as bubble-free as possible (N). Tapping firmly against the table can force large bubbles to the surface. The bath should not move easily if the container is tilted (O). Be gentle when tapping glass dishes.

N



O



It is recommended that you use a print container that provides a minimum of 1 mm clearance on the bottom and a minimum of 3 mm clearance on all sides as well as the top of the construct to be printed. Additional clearance is fine, but requires using more LifeSupport™.

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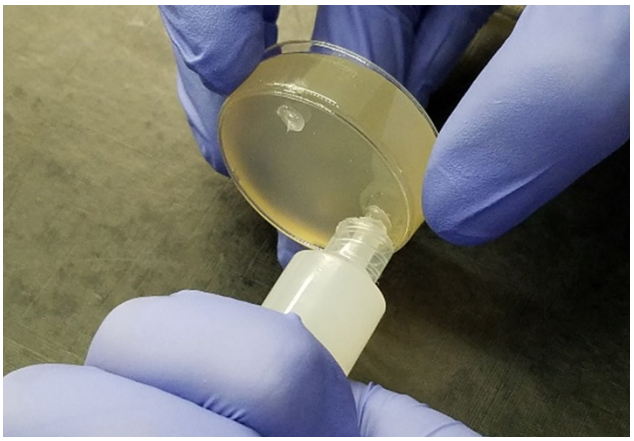
PRINTING RECOMMENDATIONS:

1. Ensure the print container is large enough to avoid the needle running into the walls during printing.

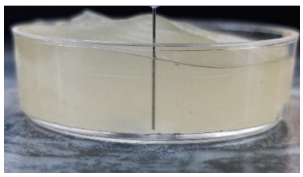
2. Place the LifeSupport™ bath in your 3D bioprinter.

   OPTIONAL Vacuum grease (Dow Corning, 1597418) can be added to the bottom of the print container to prevent sliding during printing (P).

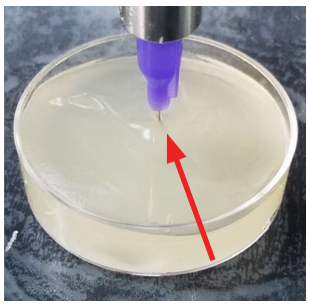


P

3. Position your needle ~1 mm off the bottom of the container (Q), then move the needle to the middle (R). Ensure that your printer begins printing from this position. You may need to disable homing procedures to prevent the printer from traveling outside of the container.



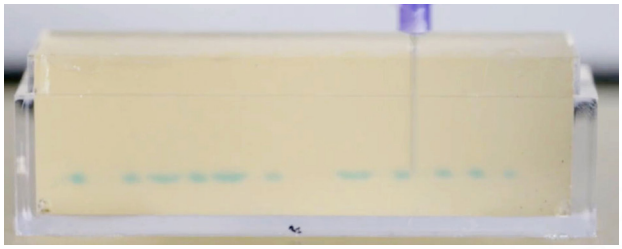
Q

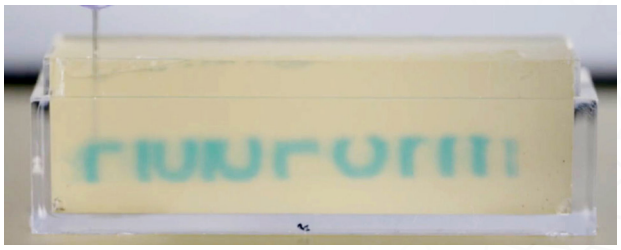


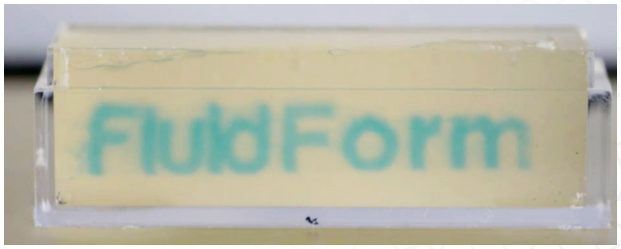
R

Unlike typical printing, the needle does not have to start out touching or even be close to the bottom of the container . The support bath will trap your print in place no matter where you start.

4. Begin printing! You are now successfully FRESH printing!







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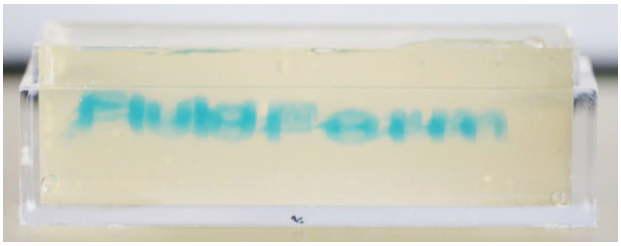
PRINT RELEASE AND RECOMMENDATIONS:

1. After printing, incubate at 37°C for at least 30 min to release your print.
2. After 30 min of incubation, the LifeSupport™ bath should be fully melted and your printed structure will be released. Large volumes may require longer times to fully melt.
3. Carefully transfer released prints into pure warm (37°C) suspension media according to your ink.

   NOTE Melted LifeSupport™ can be serially replaced with suspension media to avoid handling the printed construct. For example, if you printed into a 6-well plate, this can be done by carefully aspirating 2 mL of melted LifeSupport™ out, and adding 2 mL of warm media. Repeat this process until most of the gelatin has been replaced by media.
4. If culturing tissues, continue standard media exchange in accordance with cell culture protocol.



Released Print

Released Print in Suspension Media

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