Universal Bioink™ Kit Extrusion Bioprinting Protocol

This is a proposed extrusion bioprinting procedure with recommended printing parameters for the **Universal Bioink™**. Please adjust this protocol according to your experimental needs. To maintain the sterility of the product, work under sterile conditions. For mixing and preparation of the completely reconstituted **Universal Bioink™** see the Universal Bioink™ - Mixing Protocol (Without Cells) or the Universal Bioink™ - Mixing Protocol (With Cells) at voxcellbio.com.

Materials Required

- Completely reconstituted Universal Bioink™
- Extrusion-based bioprinter
- Magnetic stirring hotplate
- 365 or 405 nm UV light
- Bioprinter syringe
- Blunt-tip needle

Extrusion Bioprinting of Universal Bioink™

Note: the print parameters are suggestions and may vary depending on the specifications of the bioprinter being used. These instructions serve as guidelines. Please adjust the settings as needed.

If bioprinting with cells, follow the preparation of the completely reconstituted Universal Bioink™ according to the Universal Bioink™ Kit - Mixing Protocol (With Cells). For bioprinting without cells, follow the preparation of the completely reconstituted Universal Bioink™ according to the Universal Bioink™ Kit - Mixing Protocol (Without Cells).

1. Using a stirring hotplate, heat the **completely reconstituted Universal Bioink™** (containing the **Bioink Photoinitiator: LAP**) to 38 ± 2 °C while gently stirring (300-500 rpm) until a homogeneous mixture is obtained.
2. Take up the desired volume of the **completely reconstituted Universal Bioink™** using the bioprinting syringe while avoiding the introduction of air. If air has been introduced to the syringe, hold the syringe upright to allow air bubbles to flow to the top. Carefully depress the plunger to remove the undesired air.
3. Add the desired blunt-tip needle to the bioprinting syringe.
4. Load the bioprinter syringe into the bioprinter. Set the printhead to the appropriate temperature (see printing parameters table below) and allow the material to reach the desired temperature for approximately 10 minutes. The **completely reconstituted Universal Bioink™** is now ready for printing.

5. Photocrosslink the **completely reconstituted Universal Bioink™** using a UV light during and/or after extrusion bioprinting as required. If working without cells, it is recommended to use 365 nm for improved crosslinking efficiency. If working with cells, it is recommended to use 405 nm for improved cell viability. Expose the printed structure to the UV light for a minimum of 30 seconds.

   Note: the degree of crosslinking obtained using a defined exposure time will depend on the size of the construct as well as the intensity and power of the UV light.

6. After the bioprinting procedure is complete, immediately hydrate the constructs with buffer or media of choice.

Printing Parameters

The recommended printing parameters are shown below. Please adjust them to your experimental needs. The following parameters were acquired using a 365 nm UV light source to irradiate 4 combined layers of a 3D construct (4 cm by 4 cm structure with an internal grid spacing of 2.5 mm) for 30 seconds.

	Needle tip size (Gauge)	Temperature (°C)	Pressure (kPa)	Print Speed (mm/s)
Universal Bioink™	**27**	**23**	**172**	**25**