



Breast Cancer Universal Bioink™ Extrusion Bioprinting Protocol

This is a suggested extrusion bioprinting procedure with recommended printing parameters for VoxCell's Universal Bioink™, please adjust to your experimental needs. To maintain the sterility of the product, work under sterile conditions. For mixing and preparation of VoxCell's Universal Bioink™, see the *Universal Bioink™ Mixing Protocol* or the *Universal Bioink™ Mixing Protocol with Cells* at voxcellbio.com.

Materials Required

- VoxCell's Breast Cancer Universal Bioink™
- Extrusion-based 3D bioprinter
- 365 or 405 nm light
- Syringe
- Bioprinter syringe
- Female-to-female Luer lock adaptor
- Blunt-tip needle

Extrusion Bioprinting of VoxCell's Breast Cancer Universal Bioink™

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

1. Heat the **Universal Bioink™** to 37 °C (with cells) or 50 °C (without cells) using a stirring hotplate with a magnetic stir bar for 15 minutes or until any precipitated components have been fully dissolved.
2. Take up the desired volume of **Universal Bioink™** using a syringe. We suggest avoiding using a needle with the syringe for easier uptake of the bioink.
3. Hold the syringe upright so any bubbles flow to the top and carefully depress the plunger to remove any bubbles.
4. Depress the plunger on the syringe to transfer the **Universal Bioink™** to the bioprinter syringe with the use of the female-to-female Luer lock adaptor.
5. Add desired blunt-tip needle size for printing needs.
6. Load the syringe into the bioprinter. Set the printhead to the appropriate temperature (see printing parameters below) and allow the bioink to reach the desired temperature for approximately 10 minutes. The **Universal Bioink™** is now ready for printing.
7. Photocrosslink the **Universal Bioink™** using UV light either during or after extrusion bioprinting. If working without cells, we recommend using 365 nm for improved crosslinking efficiency. If working with cells, we recommend using 405 nm for improved cell viability. Expose the printed structure to 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.



Printing parameters

The recommended printing parameters are shown below, please adjust to your experimental needs. The following parameters were acquired using a 365 nm light source to irradiate a four-layer square 1 cm by 1 cm grid with spacing of 2.5 mm construct for 30 seconds.

Universal Bioink™	Syringe tip size (gauge)	Temperature (°C)	Pressure (kPa)	Print Speed (mm/s)
Breast Cancer	25	24	138	27