Universal Bioink™ Kit Digital Light Processing (DLP) Bioprinting Protocol

This is a proposed DLP 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™
- Tartrazine (100 mg in vial with magnetic stir bar)
- DLP bioprinter
- 365 or 405 nm UV light
- 1.25 mL Phosphate Buffered Saline (PBS)
- Magnetic stirring hotplate
- Pipette(s)
- Pipette tips

DLP 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.

Adding **tartrazine** (also known as Acid Yellow 23) to the **completely reconstituted Universal Bioink™** prior to DLP printing is recommended. **Tartrazine** acts as a photoabsorber, significantly increasing the resolution of DLP bioprinting.

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. Prepare a 150 mM **tartrazine stock solution** by pipetting 1.25 mL of PBS into the **VoxCell tartrazine** vial. Using a stirring hotplate, gently stir (100-200 rpm) until a homogenous mixture is obtained. Store any unused **tartrazine stock solution** at room temperature in the absence of light.

   Note: If printing in sterile conditions, use a syringe filter (0.22 µm pore size) to sterile filter the **tartrazine stock solution** prior to step 3.

2. 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.
3. Add 10 µL/mL of the **tartrazine stock solution** to the **completely reconstituted Universal Bioink™** to provide a final tartrazine concentration of 1.5 mM.
4. Transfer the **completely reconstituted Universal Bioink™** containing **tartrazine** onto the DLP bioprinter platform using a pipette. The **completely reconstituted Universal Bioink™** containing **tartrazine** is now ready for bioprinting.

   Note: Ensure that the build platform is heated to 37 °C during bioprinting to prevent thermal gelling.
5. After the bioprinting procedure is complete, immediately hydrate the constructs with a buffer or media of choice.

Printing Parameters

The recommended printing parameters are shown below. Please, adjust them to your experimental needs. Depending on the type of printhead being used, the first 2 layers may need higher exposure time and increased light intensity for build plate adhesion.

	Tartrazine Concentration (mM)	Exposure Time (s/layer)	Light Intensity (%)	Layer Height (µm)
Universal Bioink™	**1.5**	**8**	**60**	**50**