

## BIOPRINTING PROTOCOL

# PhotoHA Stiff & Soft 2%

The following protocol is a suggested procedure. Please adjust the protocol according to your own experimental needs if necessary. Both PhotoHA Stiff and Soft 2% are light-sensitive. To ensure the best possible outcome, minimize the sample's ambient light exposure and follow standard laboratory safety practices.

### Protocol aim

The aim of this protocol is to provide step-by-step instructions to print with PhotoHA Stiff and Soft 2% using BIONOVA X, including handling of the material, printing settings and safety precautions.

### Materials needed

- BIONOVA X printer \*
- PhotoHA Stiff 4% or PhotoHA Soft 4% \*
- Sterile water
- BIONOVA X 24-well plate + compatible 24-well plate probe \*
- USB drive containing STL files of desired 3D model
- Cell suspension and cell culture medium
- Sterile PBS (37 °C)
- Regular and positive displacement pipettes + tips
- Sterile lint free tissues
- Nitrile/latex gloves and lab coat

\*The product can be purchased in the CELLINK store at [www.cellink.com/store/](http://www.cellink.com/store/).

# 1. Storage, preparation and safety

- Store PhotoHA Stiff 4% and PhotoHA Soft 4% at 2-8 °C until use and protected from direct light, as the materials are light-sensitive.
- Dilute the ready to print 4% PhotoHA Stiff/Soft to 2% by adding equal amounts of ink and sterile water into a light protected vial.
- Homogenize the vial before printing by gently centrifuging or vortexing it. Let the material rest briefly to allow bubbles to settle.
- Optional (bioprinting with cells): Transfer the required cell suspension to a centrifuge tube and pellet it by centrifugation. Remove the supernatant and gently resuspend the pellet in 50–100 µL of culture medium. Add the photoink and homogenize the mixture by pipetting until cells are uniformly distributed.
- Screw the probe onto the probe holder and place it in the machine, ensuring correct alignment with the three reference lines (III) facing outward.
- Ensure printbed temperature is OFF (utility→ heater OFF) and does not exceed 37 °C.
- Wear appropriate personal protective equipment, including gloves and a lab coat, and operate the BIONOVA inside a biosafety cabinet to ensure aseptic conditions.
- If required, sterilize the interior of the machine by closing the hood and running the UV sterilization program (Utility → UV Sterilization ON) for an appropriate duration (typically 30 min for cell-based and sterile workflows); the hood must be closed for the program to initiate.

# 2. File setup (import model)

- Insert the USB drive into the BIONOVA X front USB port.
- To start a project: Select project→ New project or Open project if the project has already been created.
- If new project: Save the project on the USB or locally on the BIONOVA X.

# 3. Determine well plate settings

- Select Well plate → Choose the type of plate that you have selected for your project. Observe that this protocol is optimized for the 24-well plate.
- Proceed by selecting which wells should be included in the project. It is possible to go back to this window later and change if necessary.

# 4. Slice and load model

- Press 3D model → Load model → Choose desired stl file.
- Press Slice and confirm the dimensions of the model. Choose the number of slices. Wait until the slicing process is complete.

# 5. Parameter settings

- After the model has been sliced, choose the parameter settings by pressing Settings in the bottom panel.
- The optimized settings for PhotoHA Stiff 2% and PhotoHA Soft 2% are summarized in **Tables 1** and **2**, respectively.
- Use the parameters below as a starting point. For small deviations, the exposure parameters may be increased or decreased.

**Table 1.** Recommended starting print parameters for PhotoHA Stiff 2% using the continuous and layer-by-layer print modes.

Print mode	Sample thickness (µm)	Layer thickness (µm)	Intensity (%)	Speed (mm/s)	Time (s or s/L)
Continuous	100	-	100	0.01	-
	500	-	100	0.03	-
	1000	-	100	0.05	-
1 Layer	100-2000		80	-	10
Layer-by-layer	500	100	100	-	3
	1000	100	100	-	2.5

**Table 2.** Recommended starting print parameters for PhotoHA Soft 2% using the continuous and layer-by-layer print modes.

Print mode	Sample thickness (µm)	Layer thickness (µm)	Intensity (%)	Speed (mm/s)	Time (s or s/L)
Continuous	100	-	100	0.004	-
	500	-	100	0.02	-
	1000	-	100	0.03	-
1 layer	100-2000		80	-	25
Layer-by-layer	500	100	100	-	6
	1000	100	100	-	5*

\*This condition results in a high accumulated light dose (800mJ for 1mm print), which may have negative effects on embedded cells. While higher exposure improves print resolution, reduced exposure is generally more favorable for cell viability. When possible, users are encouraged to consider single-layer or continuous print modes to limit cumulative light exposure in cell-laden printing, or to explore the use of slightly higher PhotoHA Soft concentrations, which may allow reduced exposure while achieving sufficient crosslinking. All parameter adjustments should be optimised and validated by the user for the intended application.

**Notes:**

- The resolution is highly impacted by the fidelity of the probe. When working with viscous materials, it is highly important to clean the probe between prints to maintain high resolution prints.
- The glue on the probe tip is sensitive to strong solvents such as isopropyl alcohol (IPA). When possible, clean the probe with sterile water and wipe it off gently using sterile lint-free tissues.
- For BIONOVA X, 100% intensity corresponds to ~16 mW/cm<sup>2</sup>.
- PhotoHA concentration: This protocol and the recommended print parameters are optimized for PhotoHA Stiff and Soft 2%. Higher polymer concentrations (e.g. 4%) are more viscous and may be more challenging to distribute evenly within the well. At the same time, increased polymer concentration generally facilitates more efficient crosslinking, which may allow shorter exposure times in layer-by-layer printing or higher print speeds in continuous mode, and may enable the fabrication of stiffer and more structurally robust constructs. When working with higher concentrations, users are encouraged to carefully adjust handling and printing parameters to balance print fidelity, mechanical performance, and biological considerations for their specific application.

## 6. Loading the photoink into the well plate

- Homogenize the vial before loading the material into the well plate. This is very important as unhomogenized PhotoHA 2% does not curate accurately. The homogenization should be done before every load, but once the material has been homogenized, several wells can be loaded with the material without mixing it between sample loads.
- The amount of material needed is visible in the printing section, the last step in the panel visible in the bottom of the BIONOVA X display.

- Load material into the well by using the positive displacement pipette.
- Ensure that there are no bubbles in the well plate after loading. It is possible to load several wells at once, if PBS is added between each print (if single-print-mode is chosen). PhotoHA 2% has rather low viscosity, which reduces the need for wiping off and cleaning the probe in between, but always make sure that there is no leftover material stuck on the probe, as it impacts the resolution of the print.
- Place the well plate back into the machine by pulling back the place holder and securing it again when the plate is in the correct place.

## 7. Print execution

- To initiate printing: Select Print → Select which wells to print by choosing amongst the wells displayed in the top right corner → Press Print on top of the display.
- The printing steps taken by BIONOVA X are continuously explained during the print. It is possible to pause or stop the printing process after it has been initiated by pressing pause or stop on the display next to the print button.
- When the print is complete, wipe the probe (if necessary) and remove the excess material from the printed construct. Wash with PBS and let it sit (non-cellular studies) or wash with PBS and then remove and add cell medium instead (cellular studies).

## 8. Inspect the print

- The constructs can be visualized using a brightfield or a fluorescent microscope, depending on the nature of the experiment.

If a visible accumulation of material is present around the different features of the construct, it could be a result of over-curing/overexposure. The opposite goes for under cured constructs where the edges have low resolution instead. Any of these defects may be an indication that the parameter settings should be fine-tuned.

## 9. Store printed constructs

- Non-cellular printing: Store in fridge with PBS covering the whole constructs.
- Cellular printing: Incubate at 37 °C with medium covering the whole constructs.