

BIOPRINTING PROTOCOL

PhotoHA Stiff & Soft

The following protocol is a suggested procedure. Please adjust the protocol according to experimental needs if necessary. Both PhotoHA Stiff and Soft 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-INK Stiff and PhotoHA-INK Soft using BIONOVA X, including handling of the material, possibility to add ECM proteins, printing settings and safety precautions.

Materials needed

- [BIONOVA X printer](#)*
- [PhotoHA-INK Stiff](#) or [PhotoHA-INK Soft](#)*
- [BIONOVA X well plate](#) + [plate-compatible probe](#)*
- USB drive containing STL files of desired 3D model
- Sterile PBS
- Sterile distilled water
- Regular and positive displacement pipettes + tips
- Sterile lint free tissues
- Nitrile/latex gloves and lab coat

*The product can be purchased in the CELLINK webshop at www.cellink.com/shop/.

This protocol is organized as a step-by-step workflow: 1) material storage and preparation, 2) software setup, 3) cell-ink mixing, 4) loading the photoink into the well plate, 5) print execution, 6) print inspection and 7) storage of printed constructs.

1. Material storage and preparation

- Store PhotoHA Stiff and PhotoHA Soft refrigerated at 2–8 °C until the material preparation step. Keep the vials sealed and protected from ambient light.
- For the print conditions described in this protocol, dilute PhotoHA Stiff from 4% (original) to 2% and PhotoHA Soft from 4% (original) to 3% using sterile PBS/media of choice prior to printing. This allows addition of ECM proteins (cell-binding cites) for enhanced photoink-cell interaction. When preparing cell-laden formulations, reserve part of the dilution volume for the cell suspension added in Step 3.
- Homogenize the vial before printing by gently vortexing or by inverting the vial 5 times. Let the material rest briefly for bubbles to settle. If bubbles remain, centrifuge briefly for approximately 30 s at 1000 rpm.
- Attach the probe to the probe holder and place the holder in the machine. Ensure that the probe holder is in its correct positioning.
- PhotoHA can be printed between 18–37 °C. Select the printing temperature based on the requirements of the cells and application.
- If desired, turn on the printbed heater by selecting Utility → Heater ON → Idle.
- Select 0 s preheating and 3 mixing cycles (only for cellular printing) under Utility prior to printing
- If sterile conditions are required, run the UV sterilization program before printing by selecting Utility → UV sterilization ON.

Notes:

- For cell studies, 30 min is recommended. The hood must be closed for the sterilization program to start, and purple light is emitted during UV sterilization.
- Reserve part of the PBS dilution volume if mixing with cell suspension. For example, 4 mL of PhotoHA Stiff 2% can be prepared from 2.0 mL PhotoHA Stiff 4%, 1.9 mL PBS, and 0.1 mL cell suspension.
- The final concentration of LAP photoinitiator decreases with dilution as well. For example, in PhotoHA Stiff 2% it will be 0.25%, and in PhotoHA Soft 3% - 0.375%.
- Wear gloves and a lab coat to protect skin and clothing. Use additional PPE as required by the experimental setup and local laboratory safety procedures.

2. Software setup

2.1 Project initialization

- 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.

Note:

- When creating a new project, save it either on the USB drive or locally on the BIONOVA X before continuing with the setup.

2.2 Well plate settings

- Select Well plate → Choose the type of plate that is selected for a project.
- Proceed by selecting which wells should be included in the project.

Note:

- It is possible to return to the Well plate settings window later to modify the selected plate type or included wells if needed.

2.3 Slice and load model

- Select 3D model → Load model, then choose the desired STL file from the USB drive.
- Press Slice and confirm the dimensions of the model. Choose the number of slices.
- Wait until the slicing process is complete.

2.4 Parameter settings

- When the model has been sliced, choose the parameter settings by pressing Settings in the bottom panel.
- The recommended settings for PhotoHA-INK Stiff 2% and PhotoHA-INK Soft 3% (using 100% intensity in BIONOVA X = 16 mW/cm²) are summarized in **Tables 1** and **2**, respectively. Use the parameters below as a starting point.

Table 1. Recommended starting print parameters for PhotoHA-INK Stiff 2% using the continuous and layer-by-layer print modes. (based on acellular printing of cylinder-shaped models). Further optimization may be necessary depending on the photoink dilution (additional components), cell density (light scattering effect), model geometry, etc.

Print mode	Sample thickness (μm)	Layer thickness (μm)	Speed (mm/s)	Time (s or s/L)
Continuous	100	-	0.01	-
	500	-	0.03	-
	1000	-	0.05	-
Layer-by-layer	100-2000	100-2000 (1 layer)	-	12.5
	500	100	-	3.5
	1000	100	-	2.5

Table 2. Recommended starting print parameters for PhotoHA-INK Soft 3% using the continuous and layer-by-layer print modes. (based on acellular printing of cylinder-shaped models). Further optimization may be necessary depending on the photoink dilution (additional components), cell density (light scattering effect), model geometry, etc.

Print mode	Sample thickness (μm)	Layer thickness (μm)	Speed (mm/s)	Time (s or s/L)
Continuous	100	-	0.004	-
	500	-	0.02	-
	1000	-	0.03	-
Layer-by-layer	100-2000	100-2000 (1 layer)	-	20
	500	100	-	5
	1000	100	-	4*

*This condition results in a high accumulated light dose (640mJ 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 fewer layers or continuous print mode to limit cumulative light exposure in cell-laden printing, or to explore the use of slightly higher PhotoHA-INK Soft concentrations, which may allow reduced exposure while achieving sufficient crosslinking. All parameter adjustments should be optimized and validated by the user for the intended application.

Notes:

- If minor print deviations are observed, adjust the exposure parameters incrementally and repeat the print evaluation.
- For BIONOVA X, 100% intensity corresponds to 16 mW/cm².

3. Cell-ink mixing

- Prepare the cell suspension according to the experimental design and keep it under appropriate conditions for the selected cell type. Resuspend the cell pellet in a volume compatible with the formulation prepared in Step 1, avoiding unnecessary dilution of the photoink.
- Mix the cell suspension with the photoink gently and thoroughly using a positive displacement pipette to obtain a homogeneous cell-laden formulation while minimizing bubble formation.
- Keep the mixture protected from ambient light until loading into the well plate.

Note:

- Cell pellets should be fully resuspended before mixing with the photoink. Excessive cell suspension volumes may further dilute the formulation and may require adjustment of print parameters to maintain print quality.

4. Loading the photoink into the well plate

- Immediately before loading, homogenize the photoink or cell-ink mixture by gently inverting the vial 5 times and maintain at desired temperature until loading.
- Check the required material volume in the Printing section of the BIONOVA X interface. The volume is displayed in the final step of the bottom panel.
- Load the required amount of photoink or cell-ink mixture into the selected well using a positive displacement pipette. For cell-laden formulations, gently aspirate and dispense the mixture twice immediately before loading each well to maintain a homogeneous cell distribution.
- Confirm that no bubbles are visible in the well plate after loading.
- Place the well plate back into the machine by pulling back the clamp and securing it again when the plate is in the correct position.

Notes:

- If multiple wells are loaded before printing, keep the material protected from ambient light until use.
- Sequential printing of replicate wells without intermediate probe cleaning has been demonstrated for simple disc geometries across a full 96-well plate. Inspect the probe periodically and clean according to the probe cleaning instructions if visible residue is observed.
- Recovered material should only be reused if it remains fully liquid and free from visible gel debris. Be aware that light exposure or transfer of partially crosslinked material may affect print fidelity, resolution, or cell performance.
- Material reuse has not been broadly validated across formulations, cell types, print geometries, or applications. Users should evaluate print quality and biological performance in their own setup before implementing reuse as a routine workflow.

5. Print execution

- To initiate printing: Select Print → Select which wells to print → Press Print.
- 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.
- Remove the excess material from the printed construct and wash it with PBS for 5 minutes.
- Replace with fresh PBS (acellular studies) or cell medium (cellular studies).

Notes:

- The recommended 3 mixing cycles for cellular printing help resuspend cells prior to printing.
- Avoid routine wiping of the probe between replicate wells, as fibers may be introduced even when using lint-free tissues.

Probe use and cleaning

Maintaining a clean probe is important for achieving consistent print resolution.

Probes are consumables and may wear over time. Use of a new probe is recommended when changing photoink formulations or between users. Previously used probes may be cleaned, inspected, and stored for future reuse. Prior to reuse, inspect the probe tip and PDMS lining for visible debris, defects, or wear, as these may affect print quality.

Cleaning after printing

Clean the probe as soon as practical after printing and before residual photoink has dried on the probe surface.

- Remove the probe from the probe holder.
- If residual photoink is present, immerse the probe in DI water until the material has dissolved.
- If required, carefully remove remaining material using lint-free tissue.
- Rinse the probe with fresh DI water to remove any remaining debris or fibres generated during cleaning.
- Dry using compressed air.
- Store the probe in a clean sterile container for future use.

Probe preparation before reuse

Before reuse in cell-laden applications, users may wish to reduce bioburden on cleaned probes according to their laboratory procedures. For example:

- Immerse or rinse the probe in 70% ethanol and allow it to dry completely, or
- Place the cleaned probe with the probe tip facing upward on the BIONOVA X print bed and run a 30-minute UVC sterilization cycle.

IMPORTANT

- Photoink residues are easier to remove immediately after printing than after drying.
- Inspect the probe before reuse. Surface defects, wear, or remaining debris may affect print fidelity and surface finish.
- Replace the probe if visible defects are observed or printing performance is affected.
- When in doubt, use a new probe.

6. Inspect the print

- Inspect the printed constructs using bright-field or fluorescence microscopy, depending on the experimental setup. Check construct shape, feature definition, and the presence of visible defects before proceeding with downstream handling.

Note:

- Visible material accumulation around construct features may indicate overcuring or overexposure. Poorly defined edges or low feature resolution may indicate under-curing. If either defect is observed, fine-tune the printing parameters before repeating the print.

7. Store printed constructs

- For acellular printing, store the constructs refrigerated with enough PBS to fully cover them.
- For cellular printing, incubate the constructs at 37 °C with enough cell culture medium to fully cover them.