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BIOPRINTING PROTOCOL

# PhotoGel®-INK 50% & 95% DS

The following protocol is a suggested procedure. Please adjust the protocol according to your own experimental needs if necessary. Both PhotoGel-INK 50% and PhotoGel-INK 95% 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 PhotoGel-INK 50% and PhotoGel-INK 95% using BIONOVA X, including handling of the material, printing settings, bioprinting with cells and safety precautions.

## Materials needed

- BIONOVA X bioprinter*
- Ready-to-print PhotoGel-INK 50% or PhotoGel-INK 95%*
- BIONOVA X 24-well plate + compatible 24-well plate probe*
- USB drive containing STL files of desired 3D model
- Bead bath or other heating device
- 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/.

# 1. Storage, preparation and safety

- Store PhotoGel-INK 50% and PhotoGel-INK 95% at 2–8 °C until use and protected from direct light, as the materials are light-sensitive.
- Prior to printing, heat the photoink to 37 °C until fully liquefied and maintain this temperature throughout the entire printing process.
- Homogenize the photoink vial by gentle vortexing or by inverting it 20 times, then allow it to rest briefly to let bubbles settle. Remove remaining bubbles by centrifugation (~30 s at 1000 rpm).
- 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.
- Activate the printbed heater (Utility → Heater ON + Idle) and verify that the temperature 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 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 setting by pressing Settings in the bottom panel.
- The optimized settings for PhotoGel-INK 50% and PhotoGel-INK 95% 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 PhotoGel-INK 50% 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 | - | 70 | - | 25 |
| Layer-by-layer | 500 | 100 | 100 | - | 7 |
| | 1000 | 100 | 100 | - | 6* |

*This condition results in a high accumulated light dose (960mJ 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.

**Table 2.** Recommended starting print parameters for PhotoGel-INK 95% 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 | - | 70 | - | 15 |
| Layer-by-layer | 500 | 100 | 100 | - | 3 |
| | 1000 | 100 | 100 | - | 2.5 |

**Notes:**

- The resolution is highly impacted by the fidelity of the probe. When working with thermally gelling materials like PhotoGel-INK 50/95%, it is highly recommended to clean the probe between prints to maintain high resolution.
- The glue on the probe tip is sensitive to strong solvents such as isopropyl alcohol (IPA), etc. When possible, clean the probe with warm sterile water or PBS and wipe it off gently using sterile lint-free tissues.
- For BIONOVA X, 100% intensity corresponds to ~16 mW/cm2.

# 6. Loading the photoink into the well plate

- Homogenize the vial containing prewarmed material before printing by inverting 5 times.
- The amount of material needed is visible in the printing section, the last step in the panel visible at the bottom of the BIONOVA X display.
- Load material into the well by using the positive displacement pipette. Keep the material heated prior to loading so that it is completely liquid and easier to handle.
- Ensure that there are no bubbles in the well plate after loading. It is possible to load several wells at once if the material is kept heated, the probe is washed/wiped between prints and PBS added between each print (if single-print-mode is chosen).
- 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 position.

# 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 with lint-free tissue soaked in sterile 37 °C PBS or 37 °C sterile water.
- Remove the excess material from the printed construct and wash it with 37 °C PBS for 5 minutes.
- Replace with fresh PBS (non-cellular studies) or cell medium (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 curated 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.