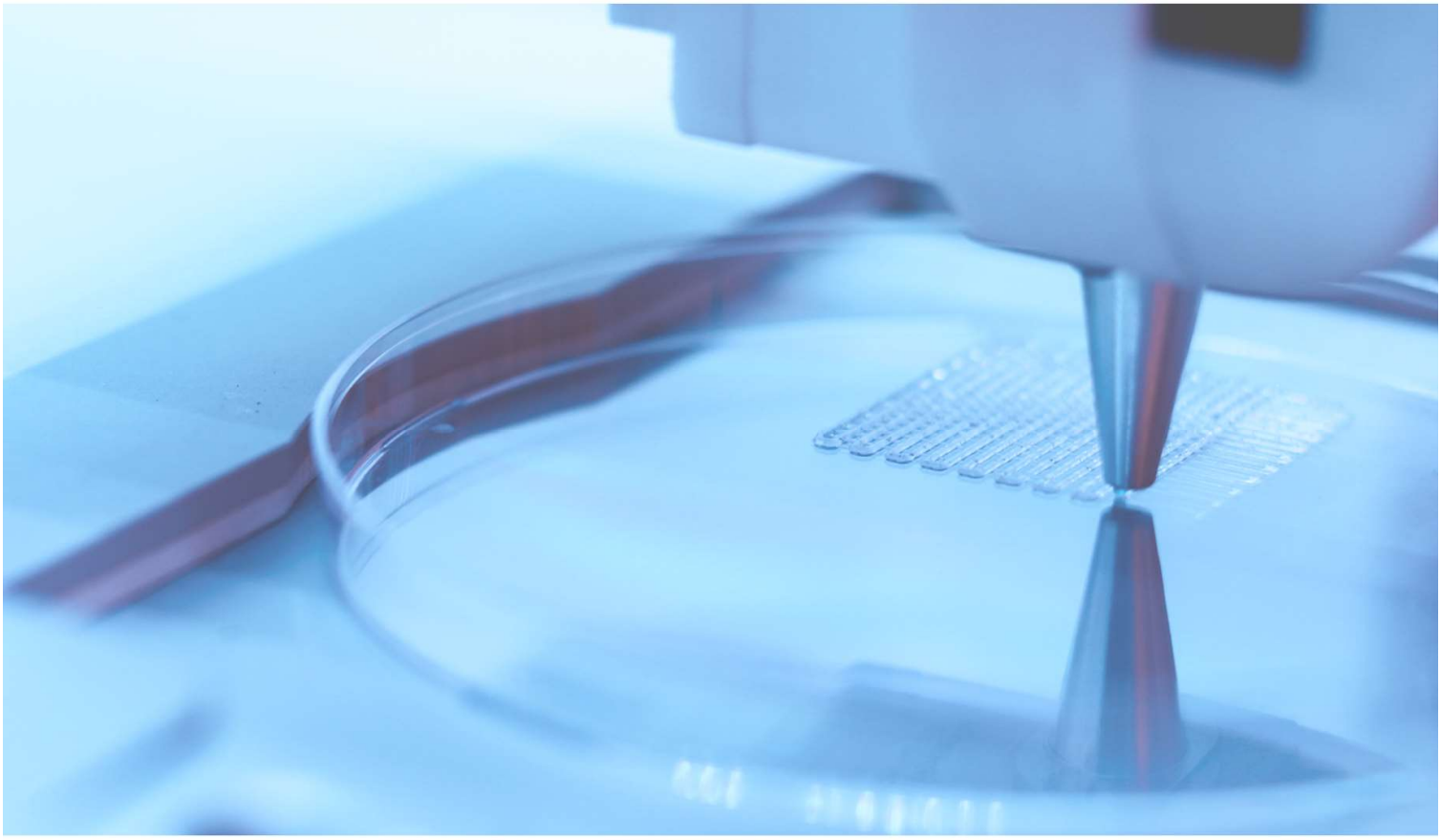
BIOINX®



User Guidelines for

HYDROBIOINX© X400

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General Information

Storage

HYDROBIO INX© X400 should be stored in a fridge until ready to use. Protect it from light. Expiry dates of the kit components are indicated on the vials and/or the sealed pouches. The products can be stored for a maximum of 3 months after opening and should be consumed before the expiry date. Always re-seal the resin vial with parafilm after use.

Intended Use

Research use only. This product is not intended for use in diagnostic or therapeutic procedures.

Safety Information

For more information, please refer to the material safety data sheet.

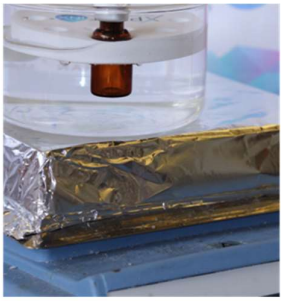
User Guidelines

Preparation

⚠ HYDROBIO INX© X400 Resin, Buffer and Crosslinker were produced under sterile conditions. To ensure optimal performance and prevent contamination, it is recommended to handle these components in a sterile environment. The provided silicon spacers and glass coverslips can be sterilized via autoclave.

⚠ The use of silanized glass substates is recommended for the attachment of the structures. For silanization, immerse the glass substrates in 3-(Trimethoxysilyl) propyl methacrylate (CAS: 2530-85-0) solution (1 v/v % in ethanol) for 45 min. Rinse thoroughly in ethanol and dry via a lens blower.

1) This kit contains one vial of HYDROBIO INX resin (1 ml), one bottle of buffer (50 ml) and 10 vials of crosslinker.



2) Place the buffer and resin vial in a water bath at 40 °C (Tip: Use the supplied floater for the resin) (±10 min)

3) Pipette correct volume[a] of buffer into the crosslinker vial. *(for volume, see footnote)*

[a] *For printing **with cells**: V_{buffer}: 0.5 ml*
*For printing **without cells**, V_{buffer}: 1 ml*

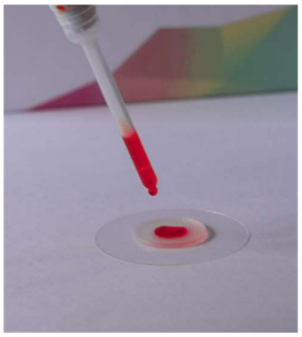
⚠ Once dissolved, the crosslinker should be used promptly and not stored in its dissolved form.

To print with cells

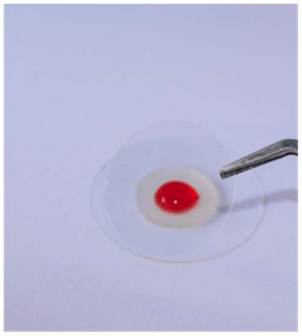
4a) Pipette 90 µl warm resin into the orange Eppendorf. Add 5 µl crosslinker and 5 µl cell suspension[b]. Gently mix to obtain homogeneous solution.[c]

To print without cells

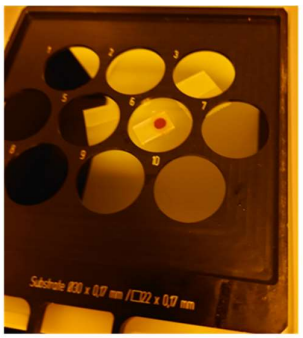
4b) Pipette 90 µl warm resin into the orange Eppendorf. Add 10 µl crosslinker. Gently mix to obtain a homogeneous solution.[c]



5) Place the mold onto the glass substrate. Take ~60 µl of the freshly prepared solution from the Eppendorf[d] and add into the mold. Avoid air bubbles.



6) Carefully place the provided cover slide on top to prevent evaporation. Gently apply pressure on top to provide proper seal.



7) Insert the substrate into the printer. If needed, apply immersion oil on the bottom of the substrate. The resin is ready to print.

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[b] *Recommended cell density: 1 - 3 million cells mL^{-1}*

[c] *It is recommended to avoid pipetting back and forth, as this can introduce air bubbles. Instead, gently stir the solution by moving the pipette tip in circular motions.*

[d] *It is recommended to warm up the Eppendorf in a water bath (37°C) prior to pipetting.*

Processing

⚠ The resin is only suitable for conventional or bottom-up printing methods.

Recommended printing parameters for 10x 0.4 NA objective:

Center Wavelength	780 nm
Repetition Rate	80 MHz
Hatching	0.5 µm
Layer Spacing	5 µm
Writing Speed	600 mm/s
Average Laser Power	> 50 mW

⚠ The printing process must be completed within 3h after preparation of the resin. The resin is not suitable for longer processing times.

⚠ If you are printing in the presence of living cells, the printing time must be limited to 1h (including preparation time). Cell printing protocol was validated using human fibroblasts. Processing parameters might vary for different cell types.

Developing

- If present, first wipe off the large part of the immersion oil from the bottom of the slide.
- Immerse the sample in warm (37°C) buffer (as supplied) or cell culture medium, and gently remove the cover slide from the sample. (If needed, wait a few minutes for the sample to warm up.)
- Keep the sample immersed up to 1 hour to allow full dissolution of the residual resin before changing the buffer or cell culture medium.

Imaging

Prior to imaging, remove the developing medium and replace with fresh buffer/cell culture medium. HYDROBIO INX X400 shows autofluorescence using an excitation wavelength of 537 nm and the emission maximum of 618 nm.

⚠ Best results are obtained when printing and imaging in glass bottom (170µm) petri dishes or cell culture chambers.