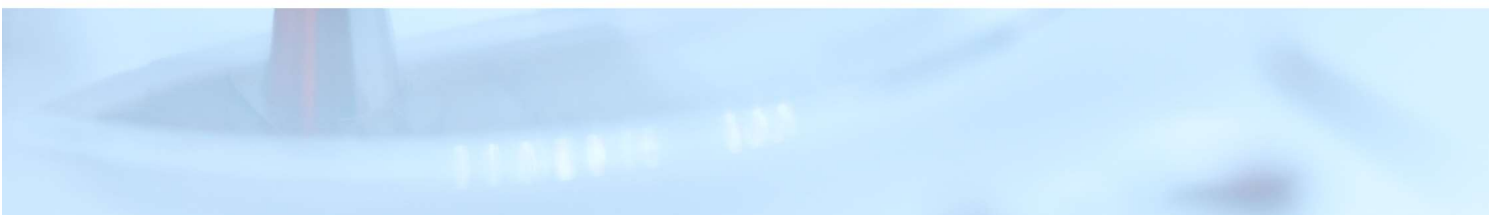


## User Guidelines for

HYDROBIO  INX<sup>®</sup> X100



## General Information

### Storage

HYDROBIO INX<sup>®</sup> X100 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 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.

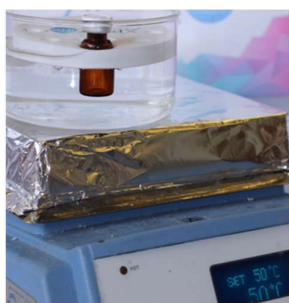
## User Guidelines

### Preparation

-  HYDROBIO INX<sup>®</sup> X100 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 substrates is recommended for the attachment of the printed 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 vial of buffer 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 1 ml warm buffer into a crosslinker vial<sup>a</sup>.



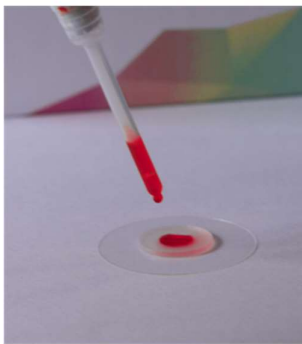
**4)** Open the warm resin vial. (Reclose the vial after use, seal with parafilm.)



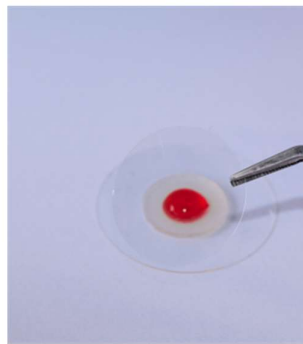
**5)** Pipette 90  $\mu$ l warm resin and 10  $\mu$ l crosslinker into the orange Eppendorf. Gently mix to obtain homogeneous solution<sup>b</sup>.



**6)** Place the supplied silicon mold onto the substrate.



**7)** Take ~60  $\mu$ l of the freshly prepared solution from the Eppendorf<sup>c</sup> and add into the mold. Avoid air bubbles.



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



**9)** 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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<sup>a</sup> Once dissolved, the crosslinker should be used promptly and not stored in its dissolved form.

<sup>b</sup> 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.

<sup>c</sup> It is recommended to warm up the Eppendorf in a water bath (37°C) prior to pipetting.

## Processing

-  HYDROBIO INX<sup>®</sup> X100 is only suitable for conventional printing or bottom-up methods.
-  The resin is not suitable for cell encapsulation.
-  The printing process must be completed within 3h after preparation of the resin. The resin is not suitable for longer processing times.

### Recommended printing parameters for 10x 0.4NA objective:

<b>Center Wavelength</b>	780 nm
<b>Repetition Rate</b>	80 MHz
<b>Hatching</b>	0.5 $\mu$ m
<b>Layer Spacing</b>	5 $\mu$ m
<b>Writing Speed</b>	600 mm/s
<b>Average Laser Power</b>	> 50 mW

## Developing

- If present, first wipe off the large part of the immersion oil from the bottom of the slide, clean remaining oil residue with a tissue and isopropanol.
- Immerse the sample in warm (37°C) buffer (as supplied) 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.

## Imaging

Prior to imaging, remove the developing buffer and replace with fresh buffer. HYDROBIO INX<sup>®</sup> shows autofluorescence using an excitation wavelength of 537 nm and the emission max of 618 nm.

-  Best results are obtained when printing and imaging in glass bottom (170 $\mu$ m) petri dishes or cell culture chambers.

## Cell Culture

The scaffolds can be readily seeded with cells after replacing the developer buffer with cell medium following overnight incubation in cell culture media without the need of additional coating.