

## User Guidelines for



## General Information

### Storage

X-PURE GEL-MA INX should be stored in a fridge at 4 °C until ready to use. Protect it from light. Expiry date of the product is indicated on the sealed pouch. The product can be stored for a maximum of 3 months after opening and should be consumed before the expiry date.

### 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



X-PURE GEL-MA INX X100 was produced under sterile conditions. To ensure optimal performance and prevent contamination, it is recommended to handle this product in a **sterile environment**.

1. Pre-heat the printhead of the 3D printer at  $28 \pm 1$  °C.
2. Remove the end-cap and tip-cap of the cartridge and attach a printing nozzle (See processing guidelines for recommended nozzle types). Insert the cartridge in the pre-heated printhead ( $28 \pm 1$  °C), and warm up until the bioink reaches the desired temperature. Depending on the used volume, it can take between 20-60 min.



For an optimal printing performance, the use of a metal conical **nozzle insulator** is required. Insert the nozzle tip in the insulator as shown in the image.



3. Calibrate the printhead and start printing using the suggested printing parameters (See processing guidelines)

## Processing

### Recommended parameters for pneumatic-based printers

Recommended processing parameters for a pneumatic-based 3D printer are listed below.

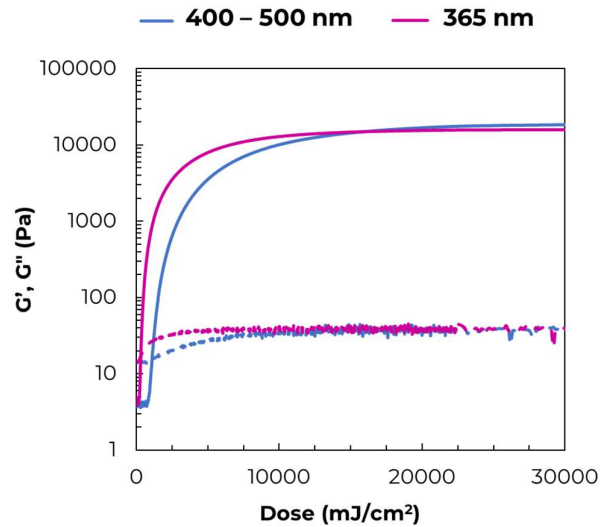
	22G Nozzle	25G Nozzle	27G Nozzle
<b>Nozzle geometry</b>	Conical	Conical	Conical
<b>Printhead Temperature</b>	28 ± 1 °C	28 ± 1 °C	28 ± 1 °C
<b>Printbed Temperature</b>	16 ± 1 °C	16 ± 1 °C	16 ± 1 °C
<b>Pressure</b>	25 ± 15 kPa	35 ± 15 kPa	40 ± 15 kPa
<b>Infill Speed</b>	8 ± 2 mm/s	8 ± 2 mm/s	8 ± 2 mm/s
<b>Layer Height</b>	0.25 ± 0.02 mm	0.18 ± 0.02 mm	0.13 ± 0.02 mm

⚠ The printing parameters have been validated for printing a scaffold with 2 mm pore diameter using 5 ml cartridges.

⚠ Use of rectilinear infill pattern is recommended.

⚠ **Photo-crosslinking:** During printing, structure should be irradiated with light ( $\lambda$ : 365 nm or 405 nm, Dose: 70 mJ/cm<sup>2</sup>) after every five layers. This step is required for partial crosslinking of the structure for a better shape retention. After completion of printing, the final structure should be promptly placed under UV light for complete crosslinking. (Recommended parameters for post-printing photo-crosslinking:  $\lambda$ : 365 or 405 nm, Dose: 10000 mJ/cm<sup>2</sup>)

For photo-crosslinking kinetics of X-PURE GEL-MA INX at two different wavelengths, see Figure 1.



**Figure 1. Storage ( $G'$ , solid lines) and loss ( $G''$ , dashed lines) moduli of X-PURE GEL-MA INX X100 as a function of irradiation dose at 365 nm and 400-500 nm wavelengths**

## Cell Culture

### a) Cell Seeding

The scaffolds can be readily seeded with cells after overnight incubation in cell culture media.

### b) Cell Encapsulation

1. Pre-heat the printhead of the 3D printer at  $28 \pm 1$  °C.
2. Pre-heat the X-PURE GEL-MA INX at 37 °C in a water bath for 10 minutes.
3. Prepare the cell suspension and insert it in a syringe with a ratio of 1:10 with respect to the volume of X-PURE Gel-MA INX.
4. Remove the end-cap and tip-cap of the cartridge.
5. Connect the X-PURE GEL-MA INX with a luer-to luer connector to the cell suspension containing syringe.
6. Inject the cell suspension into the cartridge and homogenize the cell suspension by turning the cartridge upside down and gently shaking.
7. Cool down the bioink by placing it at room temperature (21 °C) until a physical gel is formed.
8. Place the cartridge into the printhead and wait until it reaches printing temperature (10-15 min) and proceed with the printing.