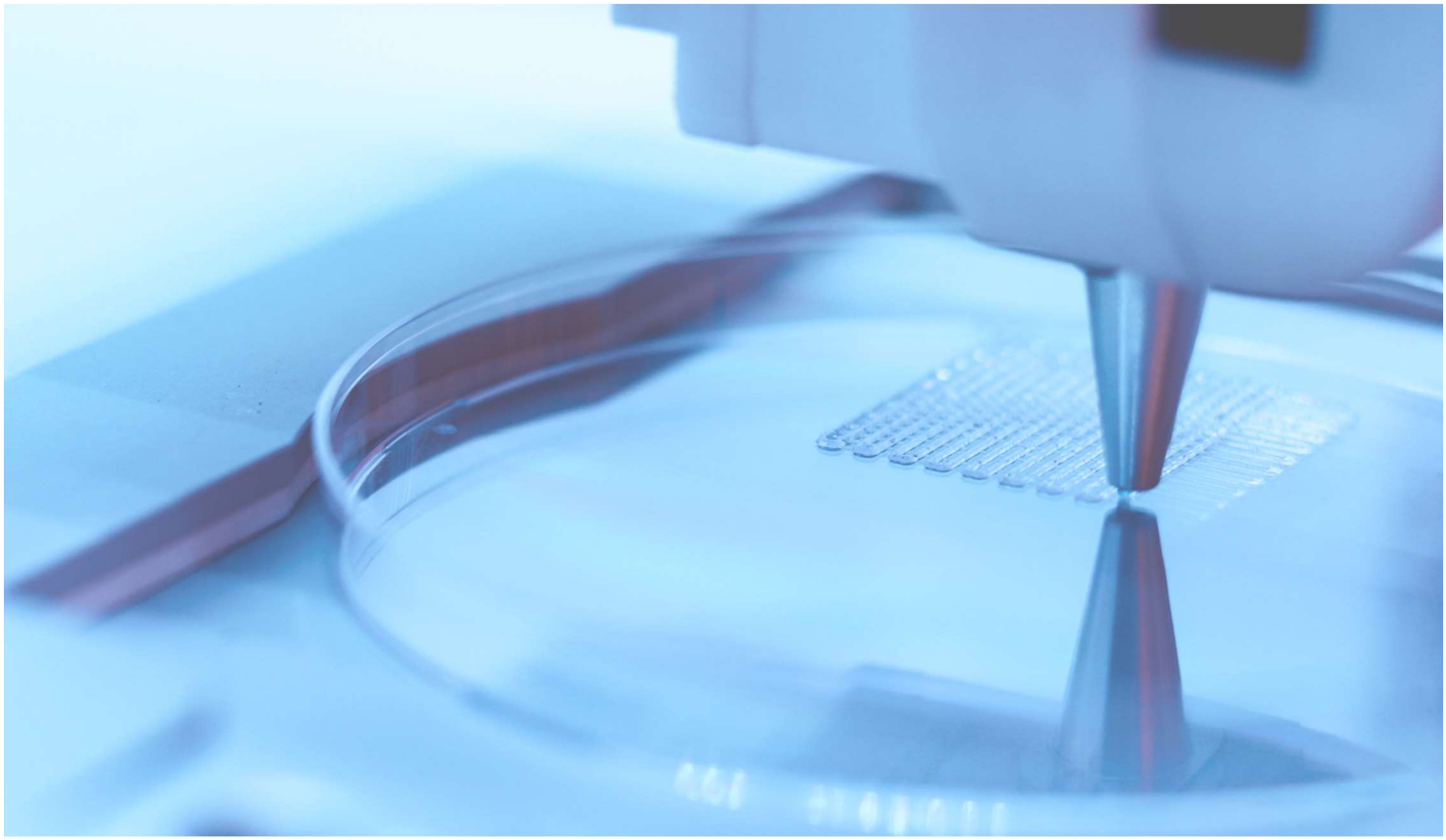
BIOINX®



# User Guidelines for

EASYGEL INX© X100

EASYGEL INX X100

# General Information

## Storage

EASYGEL INX should be stored in a fridge at 4˚C. 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

> ⚠ EASYGEL 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. Remove the end-cap and tip-cap of the cartridge. Insert a nozzle tip of choice (See processing guidelines for recommended nozzle types).
2. Pre-heat the printhead at 37 °C. Insert the cartridge in the pre-heated printhead, and warm up for at least 10 min before printing.

> ⚠ 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. Start printing using the suggested printing parameters (See processing guidelines)

## Processing

Recommended processing parameters are listed below.

| | 22G Nozzle | 25G Nozzle | 27G Nozzle |
|---|---|---|---|
| **Nozzle geometry** | Conical | Conical | Conical |
| **Printhead Temperature** | 37 °C | 37 °C | 37 °C |
| **Printbed Temperature** | 22 °C | 22 °C | 22 °C |
| **Pressure** | 40 ± 10 kPa | 50 ± 10 kPa | 50 ± 10 kPa |
| **Infill Speed** | 6 ± 1 mm/s | 6 ± 1 mm/s | 6 ± 1 mm/s |
| **Layer Height** | 0.22 ± 0.02 mm | 0.13 ± 0.02 mm | 0.08 ± 0.02 mm |

⚠ The printing parameters have been validated for printing a cube with dimensions 15 x 15 mm (W x L) using 3 ml cartridges.

⚠ Use of rectilinear infill pattern is recommended.

⚠ **Photo-crosslinking:** During printing, structure should be irradiated with light (λ: 365 nm or 405 nm, Dose: 70 mJ/cm$^2$) after every layer. 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: λ: 365 or 405 nm, Dose: 10000 mJ/cm$^2$)

For photo-crosslinking kinetics of EASYGEL INX at two different wavelengths, see Figure 1.

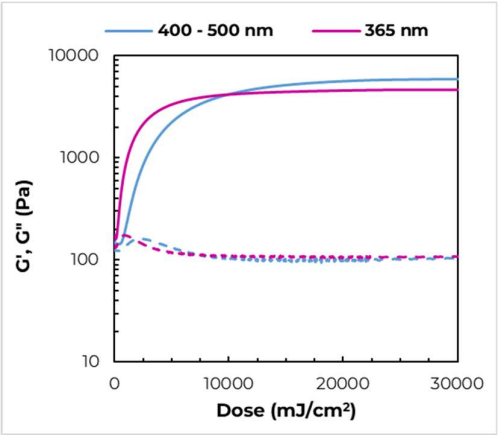



**Figure 1. Storage (G', solid lines) and loss (G", dashed lines) moduli of EASYGEL INX X100 as a function of irradiation dose at 365 nm and 400-500 nm wavelengths**

⚠ After photo-crosslinking, place the sample in a buffer or cell culture medium to prevent drying.

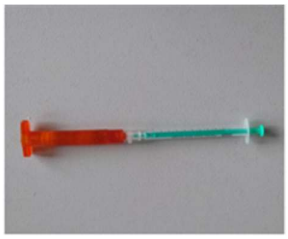
## Cell Culture

### a) Cell Seeding

The scaffolds can be readily seeded with cells after overnight incubation in cell culture media without the need of further coating.

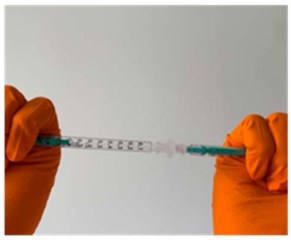
### b) Cell Encapsulation

Preheat EASYGEL INX cartridge at 37 °C.



Connect EASYGEL INX cartridge to a syringe via a female luer-to-luer adapter.

Inject the desired amount of EASYGEL INX into the syringe.



Connect the second syringe containing the cell suspension via a luer-to-luer adapter. Shift the hydrogel back and forth between the syringes until the cells are homogeneously mixed in.

Inject cell-laden EASYGEL INX back into the cartridge via a luer-to-luer adapter.

⚠ For encapsulation, the cell suspension/gel ratio should not exceed 1/10 (100 µL cell suspension per mL of final bioink).

⚠ The cell encapsulation protocol has been validated using human fibroblasts; however, it is important to note that the results obtained from this validation may vary when applied to different cell types.