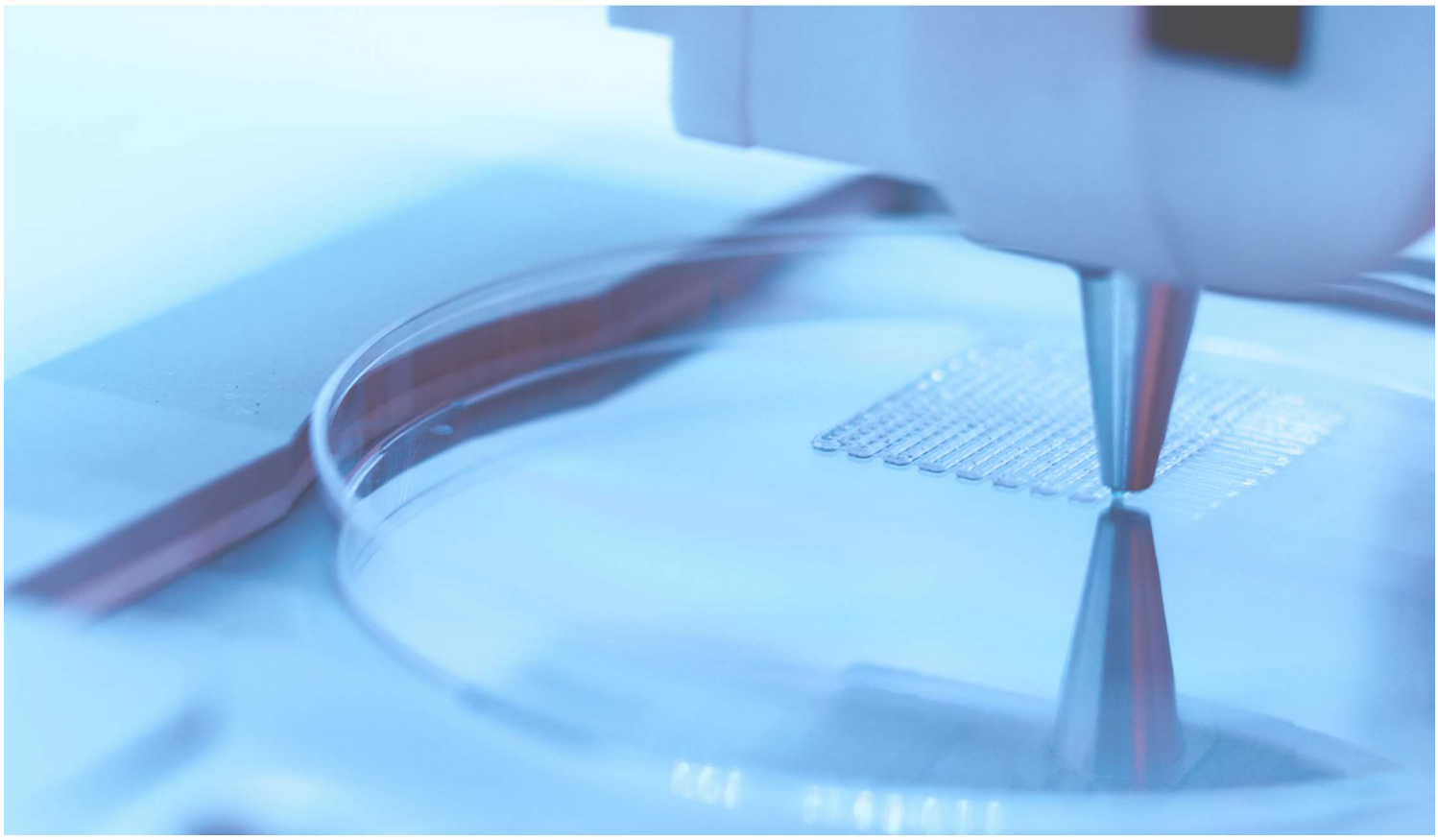
BIO INX®



User Guidelines for

READYGEL INX© X100

READYGEL INX X100

General Information

Storage

READYGEL 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 tube. 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

⚠ READYGEL 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.

Preparation

a) Without cells

1. Warm up READYGEL INX in a warm water bath at 37 °C until liquid (± 10 min).
2. Pipette the resin into suitable sample tubes for printing. Make sure to avoid air bubbles.
3. Place the sample tube in a fridge at ± 4 °C for 5 min to obtain a physical gel.
4. Place the sample tube into the printer and start printing according to the recommended processing parameters.

a) With cells

1. Warm up READYGEL INX in a warm water bath at 37 °C until liquid (± 10 min).
2. Detach the appropriate number of cells from the cell culture flask. Remove the media from the cells via centrifuge in order to obtain a cell pellet.
3. Add desired amount of READYGEL INX bioink onto the cell pellet in a Falcon tube. Gently pipette it back and forth, more than 10 times, using a pipette with a cut tip.
4. When the bioink is mixed with cells, the reference dose will be slightly different, depending on the cell type and number. In order to determine the reference dose, apply a dose test on the bioink containing cells. (see processing guidelines)
5. Pipette the resin into suitable sample tubes for printing. Make sure to avoid air bubbles.
6. Place the sample tube in a fridge at ± 4 °C for 5 min to obtain a physical gel.

7. Place the sample tube into the printer and start printing according to the recommended processing parameters.

Processing

1. Load the desired CAD model(s) for printing.
2. Start printing process following the recommended processing parameters listed below.

Refractive index	1.35
Dose*	188 mJ/cm^2
Voxel size	25 µm

* Note that the dose presented in the table refers to the reference dose obtained from the dose test. If the resin contains cells, it is recommended to apply a new dose test to determine the reference dose. The optimal dose to print a 3D structure will depend on the geometry of the CAD model and may require further adaptations.

3. After the printing process is completed, keep the sample inside the printer for 1 minute for dark curing.
4. Remove the sample tube from the printer, and place into a water bath (37 °C) to allow the resin to become liquid.

> ⚠ If the bioink contains cells, it is recommended to apply an intermediate heating step at 27 °C for 10 min, prior to immersing the vial at 37 °C.

5. Gently remove the printed structure from the ink by pouring the contents into a Petri dish.
6. Wash the printed structure(s) in warm phosphate buffer saline (PBS, 37 °C) to remove the uncrosslinked resin from the structure (± 10 min)
7. **Post-curing:** The structure should be further irradiated with light (λ: 365 or 405 nm) for complete crosslinking, while being immersed in PBS or cell culture medium.

> ⚠ If the samples contain cells, it is recommended to immerse the structure in cell culture medium without phenol red during irradiation.

   The minimum recommended dose for post-curing is 1800 mJ cm^{-2}. Immersing the sample in PBS during photo-crosslinking is required to avoid dehydration of the sample during irradiation.
8. For samples with encapsulated cells, refresh the medium, and keep the samples in a CO_2 incubator at 37 °C.
   Samples without cells can be stored in a fridge at 4 °C while being immersed in PBS.

Cell Viability Analysis

Cell viability can be evaluated using metabolic activity assays or live/dead assay to image the live and dead cells in the bioink via a confocal microscope.