

Biogelx™-INK Preparation Guidelines

Product Overview

Biogelx™-INKs offer a synthetic printable 3D material for cell culture, which mimics the native extracellular matrix. Supplied as a lyophilized powder with preparation solution, the bioinks are composed of simple, short, yet biologically relevant peptides, allowing thorough characterization and batch-to-batch reproducibility.

The recommended preparation outlined below will provide a printable bioink, capable of producing mechanically stable 3D structures. However, preparation can be modified by users to suit their needs and printer type.

Storage and Handling

- Biogelx™-INK lyophilised powders are shipped at room temperature and should be stored in a freezer at -20 °C until ready to rehydrate. The product is stable for up to 12 months when stored under these conditions.
- Biogelx™-PREP solutions are provided for the purpose of Pre-Gel preparation (e.g. 'Prep Solution A'), this will be shipped at room temperature and should be stored at 4 °C until ready for use.
- Rehydrated Biogelx™-INK is stable for a maximum of 1 month when stored at 4 °C, prior to media (or salt solution) addition.
- Bioink that has been prepared with media must be incubated for a 2 hour period at 37 °C to achieve its optimum printing viscosity.

Preparation of Biogelx™-INK

Remove vial from storage, ensure the outside of the vial is dry and allow the powder to reach room temperature before opening.

To open, remove the flip-tear-up seal and rubber stopper.

In a new sterile container, weigh the required quantity of Biogelx™-INK Powder (Table 1), this can be scaled up / down as required.

Biogelx™-INK	Bioink powder required for 1 mL bioink	Preparation of Pre-Gel	Preparation of printable Bioink
S (standard)	26.4 mg	Prep Solution A (750 µL)	Media (250 µL)
RGD	29.8 mg	Prep Solution B (450 µL)	Media (550 µL)
GFOGER	41.8mg	Prep Solution C (750 µL)	Media (250µL)

Table 1: Quantities required to prepare 1 mL of each Bioink formulation.

The printable **bioink** is prepared in two steps:

1. Preparation of the **Pre-Gel** solution using the appropriate Preparation Solution (volumes shown above).
2. Addition of media (or media + cells) to initiate cross-linking and produce the printable **Bioink** (volumes shown above).

Notes:

- If printing with cells, add 90% of the media required for cross-linking and incubate for 2 h, then add cells in the remaining 10% media immediately prior to printing.
- Bioink formulations in **Table 1** were developed using Dulbecco's Modified Eagle's Medium (DMEM).

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For new applications, it is advised that the Bioink should first be prepared following the guidelines in Table 1, before testing bespoke formulations.

1. To prepare the **Pre-Gel**, the lyophilised powder is dissolved in the required volume of Preparation Solution. Fully dissolve the lyophilised powder with mixing by vortex, and aspiration with a pipette if required (either a positive displacement pipette or an air displacement pipette with 1 cm cut from tip). It is likely that air bubbles will be present at this stage, and these can be removed by centrifugation. The material should be left to incubate overnight at 4 °C to allow peptide cooperative-assembly to occur.
2. Following overnight incubation, the printable **Bioink** is prepared by addition of media to the Pre-Gel solution. Media is added to the Pre-Gel and mixed thoroughly by vortex and aspiration with a pipette (if required). Due to the high viscosity of the Bioink, air bubbles may have formed at this point, and these can be removed by centrifugation.

Partial gelation is initiated by divalent cations in cell culture media and/or salt-containing buffers. This increases viscosity of the bioink so that extrusion printing can be performed.

Once the material has been prepared, incubate the sample at 37 °C for 2 hours to achieve the optimum viscosity for printing. Printing can then be performed at room temperature.

The bioink can be printed directly onto a flat surface at room temperature and does not require a secondary support. After printing, cell culture media can be added to increase cross linking within the material and keep the construct hydrated.

Note: If printing with cells, add 90% of the media required for cross-linking and incubate for 2 hours, then add cells in the remaining 10% media immediately prior to printing.

Product specification sheets are available on request.

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