

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 synthetically-derived peptide and polymer components, which allow thorough characterization and ensure batch-to-batch reproducibility.

The recommended preparation outlined below will produce a bioink which has been optimised for use with extrusion-based printing methods, and is capable of forming stable 3D structures without the need for another support material. Please contact a member of the team if you are interested in exploring alternative bioink formulations for use with other printer technologies.

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 item 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

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

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

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)	Medium (250 µL)
RGD	29.8 mg	Prep Solution B (450 µL)	Medium (550 µL)
GFOGER	41.8 mg	Prep Solution C (750 µL)	Medium (250 µL)

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

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Pre-Gel preparation

- Remove the Biogelx™-INK 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)
- Fully dissolve the lyophilised powder in the required volume of Preparation Solution (Table 1) with mixing by vortex and aspiration with a pipette (either a positive displacement or an air displacement pipette with 1 cm cut from tip)
- It is likely that air bubbles will form during this stage, these can be removed by centrifugation
- The material should be left to incubate overnight at 4 °C to allow peptide self-assembly to occur

Bioink preparation

- Following overnight incubation, the printable **Bioink** is prepared by addition of cell culture medium (Table 1) to the Pre-Gel solution
- The bioink should be mixed thoroughly by vortex and aspiration with a pipette
- Due to the high viscosity of the bioink, air bubbles may form and these can be removed by centrifugation
- Incubate the sample at 37 °C for 2 hours to achieve the optimum viscosity for printing

The bioink can be printed directly onto a flat surface at room temperature and does not require a secondary support. After printing, excess cell culture medium can be added to the printed structure to increase cross linking within the material and form a stable gel.

Preparation Notes:

- It is important to completely dissolve the lyophilized powder in Preparation Solution so that no lumps of powder remain. The samples can be kept at 4 °C in between mixing and centrifugation steps to aid solubility
- 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
- Bioink formulations in Table 1 were developed using Dulbecco's Modified Eagle's Medium (DMEM), which has a CaCl₂ concentration ~1.8mM. It is possible to prepare bioinks with alternative media formulations, but the CaCl₂ concentration should be similar to that found in DMEM (this can be supplemented with CaCl₂ if necessary)
- When 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
- Direct displacement pipettes are preferred for mixing viscous solutions, and these can limit the introduction of bubbles when mixing cells into the bioinks

Product specification sheets are available on request.

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