

Biogelx Powder - Preparation and Guidelines for Use in Bioprinting

Product Overview

Biogelx hydrogels offer a synthetic, tuneable 3D matrix for cells, capable of mimicking the behaviour of the body's tissues and organs. Supplied as lyophilized powder, they comprise simple, short, yet biologically relevant peptides. Our patented technology allows you to control precisely the assembly of these peptide molecules to vary the viscosity and stiffness of the gel.

The recommended preparation provided in this instruction leaflet will provide a printable Pre-Gel solution, capable of producing mechanically stable 3D structures. However, preparation can be modified by users to suit their needs and printer type.

Ways in which to achieve printed structures

- 1. Cell culture media and/or salt-containing buffers are required to trigger gelation.** To achieve a stable printed structure, printing directly into a bath of media (or media with additional calcium chloride) or calcium chloride solution is possible.
- Depending on printer type, Pre-Gel viscosity may need to be increased or decreased. This can be done by increasing or decreasing Pre-Gel concentration (**Table 1**). Optimizing this property will help improve how the material prints and help achieve structures better able to hold their shape.
- As an alternative to increasing the concentration of the Pre-Gel, the viscosity of the Pre-Gel can also be increased by the incorporation of low concentrations of crosslinking solution i.e. media or calcium chloride solution. To prepare Pre-Gel solutions in this way, we recommend using a 10 % media in water solution or a 0.5-20 mM calcium chloride solution when dissolving the Biogelx Powder.
- If a multi ink reservoir system is available that would allow for the Pre-Gel and crosslinker to combine as they are printed simultaneously, we would suggest using calcium chloride solution as the crosslinking solution (concentrations ranging from 10-100 mM have been tested in-house, to achieve the quick gelation times required. This may also be possible using media (or media with additional calcium chloride solution).

Storage and Handling

Biogelx Powder should be stored in the freezer at -20 °C until ready to rehydrate. The product is stable for up to 12 months when stored under these conditions.

Rehydrated Pre-Gel Solution is stable for a maximum of 3 months when stored at 4 °C.

Pre-Gel that has been rehydrated with salt present must be used within a 2 hour period and ideally immediately once prepared.

Preparation of Pre-Gels from Biogelx Powder

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

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

Viscosity of Pre - Gel (centipoise)	Weight of Biogelx Powder (milligrams) for 5 mL volumes
4-5	22 mg
6-7	43 mg
9-10	67 mg
100-110	110 mg
220-230	440 mg
300-310	660 mg

In a new sterile vial, weigh the required quantity of Biogelx Powder (**Table 1**).

Table 1: Weight of Biogelx Powder to prepare pre-gel of a certain viscosity.

For any new applications it is advised that Pre-Gel should be tested to optimise the procedure.

Fully dissolve the Biogelx Powder by applying vortex mixing and sonication for approximately 30 seconds. If any air bubbles are present in the Pre-Gel Solution, remove these by applying sonication to the solution for 10 seconds. (Centrifugation for 1 min (1000 rpm) can also be used to remove air bubbles)

Gelation is initiated by salt in cell culture media and/or salt-containing buffers. The material will remain in the "Pre-Gel" state until media and/or salt-containing buffer is added.

Any remaining powder can be sealed and stored at -20 °C.

If cells are to be added, they can be re-suspended/encapsulated in the Pre-Gel prior to printing. Mix well using a pipette to ensure even distribution of cells and avoid bubble formation. Cells could also be printed from a second reservoir.

Transfer the resulting Pre-Gel solution into the appropriate printer cartridge, ensuring no bubbles are introduced into the suspension.