Biogelx™ Powder - Preparation and Guidelines for Use

Preparation of Pre-Gels from Biogelx™ Powders

Cell Culture Protocol for Biogelx™ Gel

- 2D Cell Culture Method
- 3D Cell Culture Method

Addition of Growth Factors and Proteins

Contents

Materials Provided

Biogelx™ Powder packaged in a glass vial.

Storage

Biogelx™ Powder once received must be stored in the freezer at **– 20 ˚C** until ready to rehydrate with water. Biogelx™ powders are stable for up to 12 months when stored under these conditions.

After usage, store Biogelx™ Powder in the -20ºC freezer by first replacing the rubber stopper and then wrapping in Parafilm, to ensure the container is protected against moisture and air contamination.

Once rehydrated the Pre-Gel Solution is stable for a maximum of 3 months when stored at 4 °C. If solution has gone from clear/white opaque to a yellow colour, the product has expired.

Intended Use

Biogelx™ Powders are intended for research use only. This product is not intended for use in diagnostic or therapeutic procedures.

Safety Information

When working with chemicals, always wear suitable personal protective clothing. For more information, please consult the appropriate material safety data sheet (MSDS). This is available online in PDF format at www.biogelx.com.

Quality Control

In accordance with Biogelx's Quality Management System, each batch of Biogelx™ Powder is tested against predetermined specifications to ensure consistent product quality.

Product Specification sheets are available on request.

Instructions for Optimal Results with Biogelx™ Powder

GELATION

Biogelx™ gels are supplied as a lyophilized powder (Biogelx™ Powder), which is rehydrated with water to prepare a Pre-Gel solution of the user's desired concentration. This Pre-Gel is added to a well plate, along with cell culture media which promotes gelation. Cells can be cultured inside the gel (3D culture) or on top (2D culture).

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

DILUTION

When rehydrating the Biogelx™ powder, it is possible to prepare a Pre-Gel stock solution, which can then be diluted to Pre-Gels of lower concentrations. However, dilutions should only be carried out at the initial time of Pre-Gel stock preparation and not after this initial stock preparation step. This method may be employed, if the user wishes to test a wide range of final gel stiffnesses.

VISCOSITY

Pre-Gel solutions of higher concentration can be highly viscous. If the transfer of highly viscous Pre-Gels proves difficult, the use of a wide orifice pipette tip, or a pipette tip with 1 cm cut from the end is recommended.

HANDLING

Air bubbles

If any air bubbles are present in the Pre-Gel solution, remove these by placing the solution in a sonicator bath for 10 seconds or by centrifugation.

When mixing cells with the Pre-Gel solution (for 3D culture) ensure the pipette tip does not leave the Pre-Gel when mixing, as this can introduce air bubbles into the gel structure.

Media changes

Addition of media to the Pre-Gel solution should be performed **CAREFULLY**; gently pipetting cell media dropwise onto the centre of the Pre-Gel. Pipetting down the side of the well can disrupt the material.

Lower concentration of Pre-Gel solutions will have lower viscosities and may appear almost water-like in a vial. However, when added to a well/insert the surface tension of the Pre-Gel ensures that it is not disrupted by/mixed with the media.

It is necessary to handle the material very carefully when performing media changes. Do not use a vacuum aspirator to remove media from above the hydrogel. Avoid direct contact with the hydrogel.

Preparation of Pre-Gels from Biogelx™ Powders

Remove the glass vial containing Biogelx™ Powder from freezer, 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.

Under sterile conditions; ie. Under a class II laminar flow hood and in a new sterile vial, weigh the required quantity of Biogelx™ Powder. Weights for Biogelx™ Powder range are shown in Tables 1 – 5.

For any new applications, it is advised that a range of stiffnesses should be tested to optimise the procedure.

Biogelx™ Standard Powder

Table 1: Weight of **Biogelx-S** to prepare Gels of a certain stiffness.

Stiffness range of Gel required (kPa)	Weight of **Biogelx–S** (milligrams) for 1 mL volumes.
0.5 – 1.5	4.4 mg
2.0 – 4.5	8.8 mg
6.0 – 10.0	13.2 mg

Biogelx™ Fibronectin Powder

Table 2: Weight of **Biogelx–RGD** to prepare Gels of a certain stiffness.

Stiffness range of Gel required (kPa)	Weight of **Biogelx–RGD** (milligrams) for 1 mL volumes.
0.5 – 2.5	5.0 mg
5.5 – 8.0	9.9 mg
9.0 – 15.0	14.9 mg

Biogelx™ Collagen Powder

Table 3: Weight of **Biogelx–GFOGER** to prepare Gels of a certain stiffness.

Stiffness range of Gel required (kPa)	Weight of **Biogelx–GFOGER** (milligrams) for 1 mL volumes.
0.5 – 2.5	5.8 mg
4.5 – 7.0	11.6 mg
7.5 – 10.5	17.4 mg

Biogelx™ Laminin Powders

Table 4: Weight of **Biogelx-IKVAV** to prepare Gels of a certain stiffness.

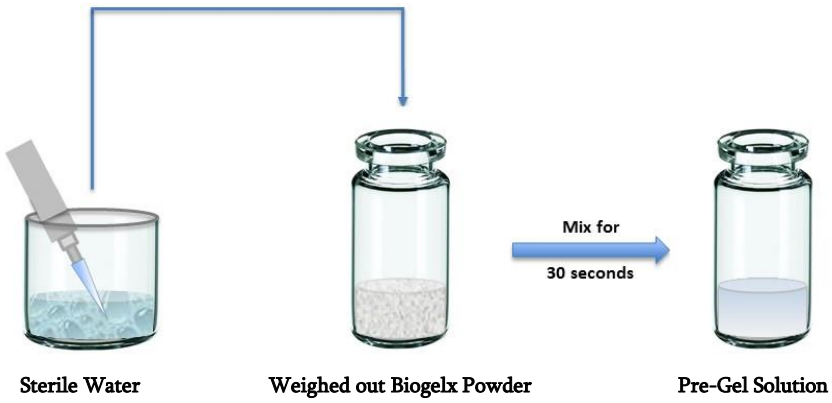
Stiffness range of Gel required (kPa)	Weight of **Biogelx–IKVAV** (milligrams) for 1 mL volumes.
0.8 – 1.1	4.5 mg
3.0 – 4.0	8.9 mg
8.0 – 9.2	13.4 mg

Table 5: Weight of **Biogelx–YIGSR** to prepare Gels of a certain stiffness.

Stiffness range of Gel required (kPa)	Weight of **Biogelx–YIGSR** (milligrams) for 1 mL volumes.
0.8 – 1.1	5.6 mg
3.0 – 4.0	11.1 mg
8.0 – 9.2	16.7 mg

Store the unused Biogelx™ Powder in the -20ºC freezer (see Storage section).

Ensure that all of the weighed out Biogelx™ Powder is located at the bottom of the vial and then carefully pipette the required volume of sterile ultrapure water to prepare the Pre-Gel Solution. **Volume of Pre-Gel Solution prepared can be scaled up or down as required.**



Fully dissolve the Biogelx™ Powder by applying vortex mixing and sonication for approximately **30 seconds** each.

Store the Pre-Gel Solution at 4 °C until required for cell culture.

NOTE: Bubbles must be avoided when using gels for cell culture methods.

If bubbles are still present in the Pre-Gel Solution after the first step of sonication, apply another step of sonication to the solution for 10 more seconds and/or leave the Pre-Gel Solution in the fridge at 4ºC overnight to help remove bubbles.

Cell Culture Protocol for Biogelx™ Gels in 2D and 3D

Pre-Gel Preparation for 2D and 3D Cell Culture:

NOTE: The Pre-Gel Solution can be sterilized under UV light for 30-40 minutes before use to eliminate further chances of contamination.

Prior to use, place the cell media and Pre-Gel solutions in an incubator or water bath until the solution(s) reaches 37 °C (around 30 minutes).

NOTE: It is advisable to incubate only the required volume of Pre-Gel solution needed for the experiment.

Remove the solution(s) from the incubator/water bath and GENTLY mix the Pre-Gel solution using a pipette to produce a homogeneous solution. During mixing the pipette tip should not be removed from the solution. If air bubbles are present in the solution, remove these by placing the solution in a sonicator bath for 10 seconds.

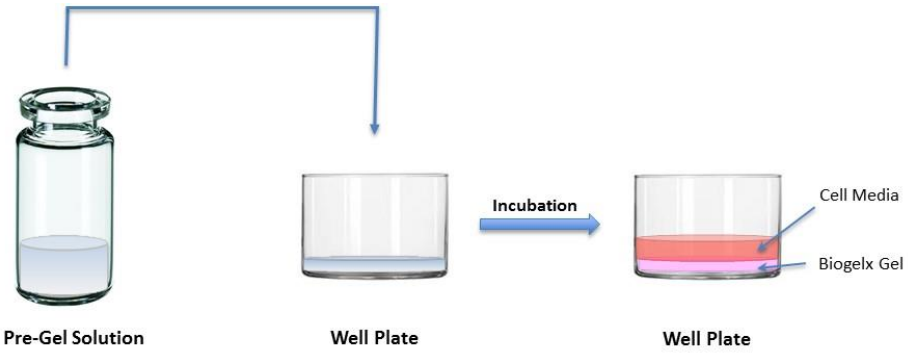
Proceed with 2D and/or 3D cell culture methods independently

2D Cell Culture Method

2D Cell Culture Method (Well Format)

Gel Preparation (Well Format):

Gently pipette the Pre-Gel solution into the bottom of the well plate. Volumes of Pre-Gel solution required are shown in **Table 6** for each plate size used. **Refer to diagram below for guidance.**



Place the well plate containing the Pre-Gel solution in the cell incubator at 37 °C with a humidified atmosphere of 5 % CO_2 for the time specified in **Table 6**.

NOTE: At this stage, gelation has not occurred.

To promote gelation, **GENTLY** pipette cell media dropwise onto the centre of the Gel (refer to **Table 6** for cell culture media volumes), making sure the pipette tip does not touch the surface of the Gel whilst doing so. The Pre-Gel and media should form two distinct layers (**depicted above**).

Place the culture plate in the incubator for a **minimum of 2 hours**. During this time, the media will diffuse through the Pre-Gel promoting gelation.

Carefully change cell culture media added to the surface of the Gel. Place the plate back in the incubator and leave overnight.

NOTE: To avoid direct contact with the Gel, carefully place the pipette tip at the top of the media and remove the media slowly. While changing media, remember to gently pipette dropwise onto the centre of the Gel making sure the pipette tip does not touch the surface of the Gel.

Table 6: Volumes of Pre-Gel solution and Media required for well plate sizes performing 2D cell culture in well format.

Culture Plate Size	Minimum volume of Pre-Gel solution per Well (µL)	Incubation time before media addition (minutes)	Volume of Cell Culture Media (µL) onto surface of Gel
96 Well	100	15	150
48 Well	200	15	250
24 Well	500	30	600
12 Well	800	30	1000
6 Well	3000	60	4000

Cell seeding on top of the gels for 2D culture (Well Format):

Carefully change cell culture media added to the surface of the Gels.

Centrifuge the trypsinised cells from your flask/dish, change media and re-suspend the cell pellet in fresh media to give a single cell suspension. Determine cell density. Prepare an appropriate concentration of cells in cell culture media (typically **4 x 10^4 – 5 x 10^5 cells/cm^2** final concentration for most cell types).
Refer to **Table 7** for details.

NOTE: The cell number stated is a recommended guideline only and can be adjusted depending on the cell type or application.

Remove the old media from the Gel surface. Gently pipette the prepared cell suspension onto the top of the Gel.

Table 7: Concentrations and Volumes of Cell Suspension required for well plate sizes using 2D cell culture in well format.

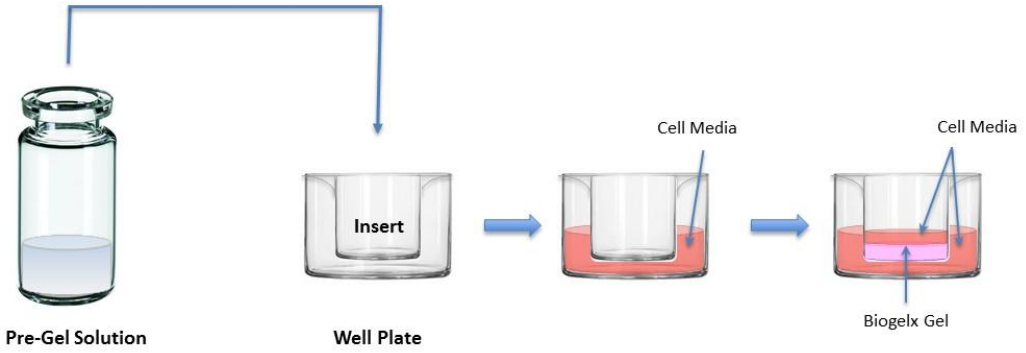
Culture Plate Size	Volume of Prepared Cell Suspension (µL) on top of Gel
96 Well	150
48 Well	250
24 Well	600
12 Well	1000
6 Well	4000

Media should be replaced with fresh media every 24 hours during the first two days of incubation, and every second day after that.

2D Cell Culture Method (Insert Format)

Gel Preparation (Insert Format):

GENTLY pipette the required volume of Pre-Gel solution into each well plate insert. Volumes of Pre-Gel solution and cell culture media required are shown in **Table 8** for each plate size. Add cell media into each well **outside of the insert**, so that the Pre-Gel solution in each insert is in contact (through the membrane) with media from underneath. **Refer to diagram below for guidance.**



Place the culture plate containing the inserts, add media to outside of each insert and Pre-Gel solution in the cell incubator at 37 °C with a humidified atmosphere of 5 % CO_2 for **30 minutes**.

NOTE: At this stage, complete gelation has not occurred.

To promote gelation, **GENTLY** pipette cell media dropwise onto the centre of the Gel, making sure the pipette tip does not touch the surface of the Gel whilst doing so. Refer to **Table 8** for cell culture media volumes.

Place the culture plate in the incubator for a **minimum of 2 hours** before adding cells. During this time, the media will diffuse through the Pre-Gel promoting gelation.

Carefully change cell culture media added outside the insert and to the surface of the Gel (preferably following this order). Place the plate back in the incubator and leave overnight.

NOTE: To avoid direct contact with the gel, tilt the insert and carefully place the pipette tip at the top of the media and remove the media slowly. While changing the media on top of the gel, remember to gently pipette dropwise onto the centre of the Gel making sure the pipette tip does not touch the surface of the Gel.

Table 8: Volumes of Pre-Gel solution and Cell Culture Media required for well plate sizes performing 2D cell culture with inserts.

Culture Plate Size	Volume of Pre-Gel per Insert (µL)	Volume of Cell Culture Media (µL) added outside the insert	Volume of Cell Culture Media (µL) added to surface of Gel
24 Well	100	1000	100
12 Well	300	1400	300
6 Well	1000	2000	1000

Cell seeding on top of gels for 2D cell culture (Insert Format):

Carefully change cell culture media added outside the insert and to the surface of the gel.

Centrifuge the trypsinised cells from your flask/dish, change media and re-suspend the cell pellet in fresh media to give a single cell suspension. Determine cell density. Prepare an appropriate concentration of cells in a final volume (typically **4 x 10^4 – 5 x 10^5 cells/cm^2** final concentration for most cell types).
Refer to **Table 9** for details.

NOTE: The cell number stated is a recommended guideline only and can be adjusted depending on the cell type or application.

Remove the old media from the Gel surface within the insert. **GENTLY** pipette the prepared cell suspension onto the top of the gel in each insert.

Table 9: Concentrations and Volumes of Cell Suspension required for well plate sizes using 2D cell culture with inserts.

Culture Plate Size	Volume of prepared Cell Suspension (µL) on top of Gel
24 Well	100
12 Well	300
6 Well	1000

Media should be replaced with fresh media every 24 hours during the first two days of incubation, and every second day after that.

3D Cell Culture Method

Cell Seeding and Pre-Gel Preparation for the 3D Culture Method:

Centrifuge the trypsinised cells from your flask/dish, change media and resuspend the cell pellet in fresh media to give a single cell suspension. Determine cell density. Prepare an appropriate concentration of 0.5 – 1 x 10^6 cells/ml in a final volume of cell culture media.

NOTE: The volume of cell culture media must equal the Pre-Gel Solution volume to be used. The volume of Pre-Gel Solution depends on the 3D culture method to be applied (well-, insert- or spheroid-formats). Refer to **Tables 10, 11 and/or 12** for details.

Centrifuge the cell-media mix for 5 minutes at 1500 rpm to obtain a cell pellet and discard media (leave a maximum of 10% media). Disturb the pellet through heavy shaking to allow breaking up of the cell mass.

Add the required volume of Pre-Gel Solution for a concentration of 0.5 – 1 x 10^6 cells/ ml.

Mix carefully pipetting up and down to allow even distribution of cells in the Pre-Gel Solution. Ensure the pipette tip does not leave the Pre-Gel solution when mixing as this can introduce air bubbles into the Gel structure.

3D Cell Culture Method

Plating the Pre-Gel Solution including cells:

3D Cell Culture Method (Well Format)

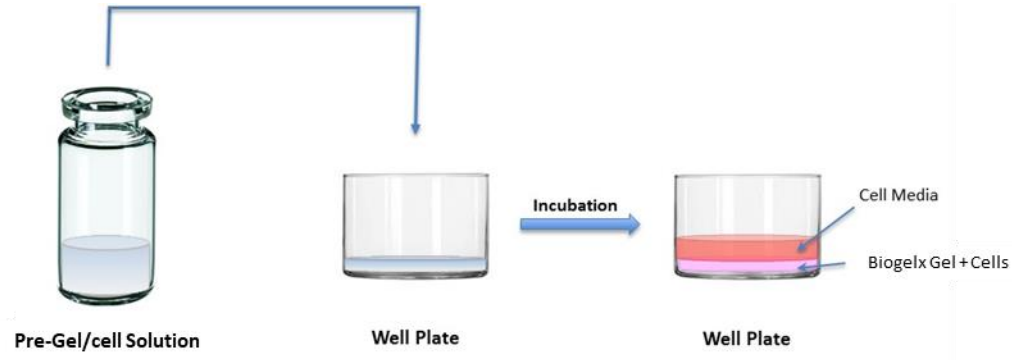
GENTLY pipette the prepared Pre-Gel/Cell Solution into the bottom of the well plate required. Volumes required for each plate size used are shown in **Table 10**. **Refer to diagram below for guidance.**

Table 10: Volumes of Pre-Gel/Cell Solution and Cell Culture Media required for well plate sizes performing 3D cell culture in well format.

Culture Plate Size	Minimum volume of Pre-Gel/Cell Solution per Well (µL)	Volume of Cell Culture Media added to the Gel surface (µL)
96 Well	100	150
48 Well	200	250
24 Well	500	600
12 Well	800	1000
6 Well	2500	3000

Place the culture plate containing the Pre-Gel/Cell Solution in the cell incubator at 37 °C with a humidified atmosphere of 5 % CO_2 for **30 minutes**.

NOTE: At this stage, gelation has not occurred.



To promote complete gelation, **GENTLY** add media dropwise onto the centre of the Gel, making sure the pipette tip does not touch the surface of the Gel whilst doing so (refer to **Table 10** for cell culture media volumes). The Pre-Gel/Cell Solution and media should form two distinct layers (**depicted above**).

Plate the culture plate in the incubator for a minimum of 2 hours. During this time, the media will diffuse through the Pre-Gel/Cell Solution promoting gelation.

Replace the media on the surface of the gel after **2 hours** with fresh media, place the plate back in the incubator and leave overnight.

NOTE: Care should be taken when removing the media on top of the gel; to avoid direct contact with the gel, carefully place the pipette tip at the top of the media and remove the media slowly. While replacing

the media, remember to gently pipette dropwise onto the centre of the Gel making sure the pipette tip does not touch the surface of the Gel.

Media should be replaced with fresh media every 24 hours during the first two days of incubation and every second day thereafter.

3D Cell Culture Method (Insert Format):

GENTLY pipette the prepared Pre-Gel/Cell Solution to each well plate insert required. Add cell culture media into the well (outside the insert) so that the Pre-Gel/cell Solution in each insert is in contact (through the membrane) with media from underneath. Refer to diagram below for guidance. Volumes for Pre-Gel/Cell Solution and media for each culture plate size are shown in **Table 11.**

Place the culture plate containing the inserts, add media to outside of each insert and Pre-Gel/Cell Solution in the cell incubator at 37ºC with a humidified atmosphere of 5% CO2 for **30 minutes.**

NOTE: At this stage, gelation has not occurred.

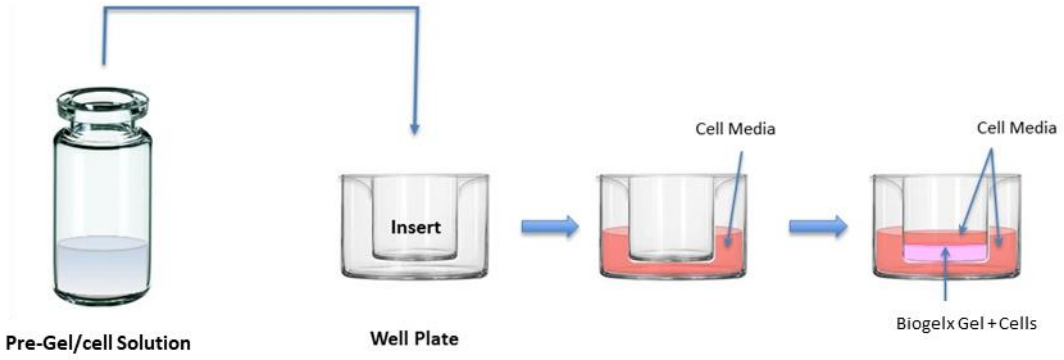


Table 11: Volumes of Pre-Gel/Cell Solution and Cell Culture Media required for performing 3D cell culture with inserts.

Culture Plate Size	Volume of Pre-Gel/Cell Solution per Insert (µL)	Volume of Cell Culture Media (µL) outside the insert	Volume of Cell Culture Media added to the gel surface (µL)
24 Well	100	1000	100
12 Well	300	1400	300
6 Well	1000	2000	1000

GENTLY pipette cell media dropwise onto the centre of the Gel, making sure the pipette tip does not touch the surface of the Gel whilst doing so (refer to **Table 11** for cell culture media volumes).

Place the culture plate in the incubator for a minimum of 2 hours. During this time, the media will diffuse through the Pre-Gel/Cell Solution promoting gelation.

Carefully replace the media surrounding the insert and on the surface of the Gel (**Table 11**, preferably in this order) with fresh media, place the plate back in the incubator and leave overnight.

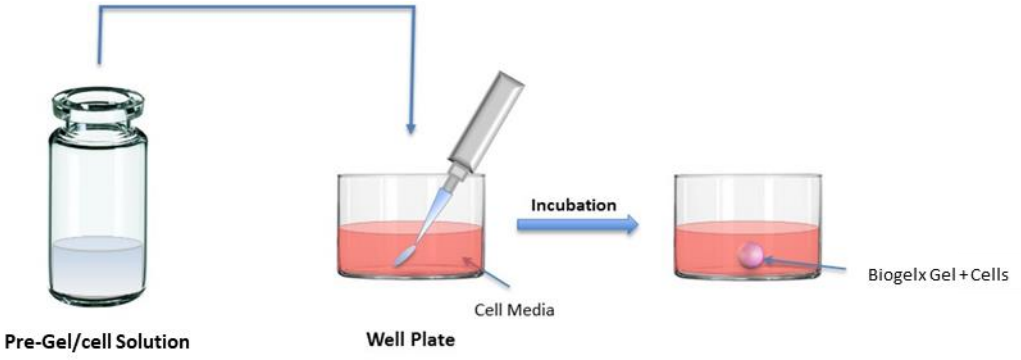
NOTE: to avoid direct contact with the Gel, tilt the insert, carefully place the pipette tip at the top of the media and remove the media slowly. While replacing the media on top of the Gel, remember to gently pipette dropwise onto the centre of the Gel making sure the pipette tip does not touch the surface of the Gel.

Media should be replaced with fresh media every 24 hours during the first two days of incubation and every second day thereafter.

3D Cell Culture Method (Sphere Format)

Add cell media to each of the desired wells in the required well plate (**Table 12** shows volume required for each well plate size).

Then **GENTLY** add the Pre-Gel/Cell Solution to each well, using a pipette **in which the tip has had 1 cm of the end cut from it.** This allows for the Pre-Gel/Cell Solution to be delivered to the well in a ball-like structure. Pipette tip should be fully immersed before Pre-Gel/Cell Solution is released into the media, and the plunger of the pipette depressed in one smooth motion to form a single gel "sphere" - **do not add drop-wise**. **Refer to diagram below for guidance.**



Incubate the prepared well plates at 37 °C with a humidified atmosphere of 5 % CO_2 for **1 hour**. During this time, the media will diffuse through the Pre-Gel/Cell Solution promoting gelation.

Carefully replace the media surrounding the sphere with fresh media (**Table 12**), place the plate in the incubator and leave overnight.

Table 12: Volumes of Cell Culture Media and Pre-Gel/Cell Solution required for performing 3D cell culture in sphere format.

Culture Plate Size	Volume of Cell Culture Media (µL) in the well	Volume of Pre-Gel Cell Solution per well (µL)
96 Well	150	50
48 Well	500	100
24 Well	1500	300
12 Well	2000	300

Media should be replaced with fresh media every 24 hours during the first two days of incubation and every second day thereafter. Care should be taken when removing the media, to avoid direct contact with the Gel.

Addition of Growth Factors and Proteins

To Biogelx™ Pre-Gel Solution

Reconstitute the additional growth factor/protein as directed by its product guidelines.

Pre-mix the required volume of additional growth factor/protein with cell culture media to obtain the final working concentration.

Prepare the Gel following the required Cell Culture Method for Biogelx™ Gels in 2D and/or 3D.

To Biogelx™ Powder

If suitable, growth factor/protein can be weighed out and directly added to the weighed out Biogelx™ Powder prior to Pre-Gel preparation.

If growth factor/protein is required to be prepared separately and is already in solution this can then be used to reconstitute the Biogelx™ Powder in the Pre-Gel preparation step instead of sterile water.

Proceed as described in the Cell Culture Protocol for Biogelx™ Gels in 2D and/or 3D.

To repeat your order please visit www.biogelx.com

For technical support please call +44 (0) 1698 534655 or contact us at info@biogelx.com.

Stay tuned! Follow us on Twitter and LinkedIn.

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