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

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Materials Provided

Biogelx Powder packaged in a glass vial.

Storage

Biogelx Powder should 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.

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, 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 a suitable personal protective clothing. For more information, please consult the appropriate material safety data sheet (MSDS). This 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.

Hints 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 higher concentration Pre-Gel stock solution, which can be diluted to Pre-Gels of lower concentrations. **However, dilutions should only be carried out at the time of Pre-Gel preparation and not in the future**. 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 tips, 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 bath sonicator 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

First 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 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 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.

In a new sterile vial, weigh the required quantity of Biogelx Powder. Weights for Biogelx Powder range and shown in Tables 1 – 6.

NOTE: Should a gel be required that is of lower or higher stiffness than those detailed in the table, the quantity of Biogelx Powder may be scaled appropriately at the user's discretion.

For any new applications, it is advised that each stiffness of gel should be tested to optimise the procedure

Standard Biogelx Powder

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

Stiffness range of Gel required (kPa)	Weight of Biogelx Powder (milligrams) for 5 mL volumes.
0.8 – 1.1	22 mg
3.0 – 4.0	43 mg
8.0 – 9.2	67 mg

Biogelx Laminin Powders

Table 2: Weight of **Biogelx Powder – Laminin IKVAV** to prepare Gels of a certain stiffness.

Stiffness range of Gel required (kPa)	Weight of Biogelx Powder – Laminin IKVAV (milligrams) for 5 mL volumes.
0.8 – 1.1	26 mg
3.0 – 4.0	52 mg
8.0 – 9.2	79 mg

Table 3: Weight of **Biogelx Powder – Laminin YIGSR** to prepare Gels of a certain stiffness.

Stiffness range of Gel required (kPa)	Weight of Biogelx Powder – Laminin YIGSR (milligrams) for 5 mL volumes.
0.8 – 1.1	30 mg
3.0 – 4.0	61 mg
8.0 – 9.2	91 mg

Biogelx RGD Powders

Table 4: Weight of **Biogelx Powder – RGD standard** to prepare Gels of a certain stiffness.

Stiffness range of Gel required (kPa)	Weight of **Biogelx Powder – RGD std** (milligrams) for 5 mL volumes.
0.8 – 1.1	25 mg
3.0 – 4.0	50 mg
8.0 – 9.2	74 mg

Table 5: Weight of **Biogelx Powder – RGD low** to prepare Gels of a certain stiffness.

Stiffness range of Gel required (kPa)	Weight of **Biogelx Powder – RGD low** (milligrams) for 5 mL volumes.
0.8 – 1.1	23 mg
3.0 – 4.0	46 mg
8.0 – 9.2	70 mg

Table 6: Weight of **Biogelx Powder – RGD high** to prepare Gels of a certain stiffness.

Stiffness range of Gel required (kPa)	Weight of **Biogelx Powder – RGD high** (milligrams) for 5 mL volumes.
0.8 – 1.1	27 mg
3.0 – 4.0	53 mg
8.0 – 9.2	79 mg

Biogelx Collagen Powders

Table 1: Weight of Biogelx Powder – GFOGER to prepare Gels of a certain stiffness.

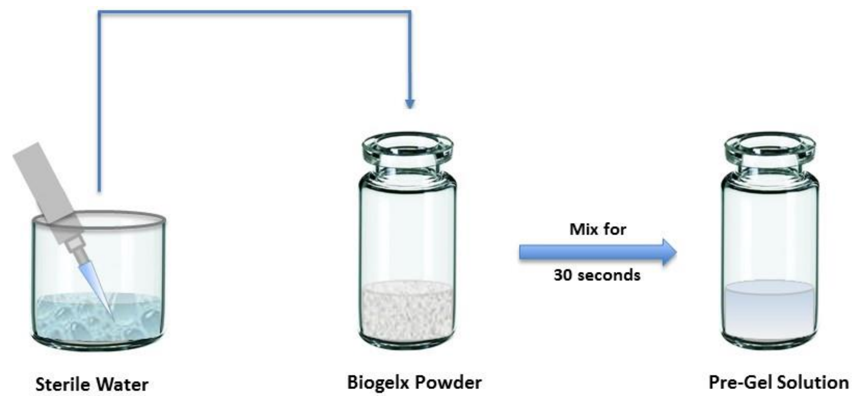
Stiffness range of Gel required (kPa)	Weight of Biogelx Powder – GFOGER (milligrams) for 5 mL volumes.
0.8 – 1.1	26 mg
3.0 – 4.0	52 mg
8.0 – 9.2	78 mg

Biogelx Lactobionic Acid Powders

Table 1: Weight of Biogelx Powder – LBA to prepare Gels of a certain stiffness.

Stiffness range of Gel required (kPa)	Weight of Biogelx Powder – LBA (milligrams) for 5 mL volumes.
0.8 – 1.1	22 mg
3.0 – 4.0	43 mg
8.0 – 9.2	65 mg

Ensure that all of the Biogelx Powder is located at the bottom of the vial and then carefully pipette **5 mL** of sterile 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**. If any air bubbles are present in the Pre-Gel Solution, remove these by applying sonication to the solution for **10 seconds**.

Store the Pre-Gel Solution at 4 °C until required for cell culture. The Pre-gel may foam on mixing by vortex, if so leave the Pre-Gel Solution in the fridge at 4 ˚C overnight before use in cell culture methods.

Replace the unused Biogelx Powder in the -20 ˚C freezer by first replacing the rubber stopper and then wrapping in Parafilm or similar material to ensure the container is protected against moisture and air contamination.

Cell Culture Protocol for Biogelx Gel

2D CELL CULTURE METHOD

Pre-Gel Preparation

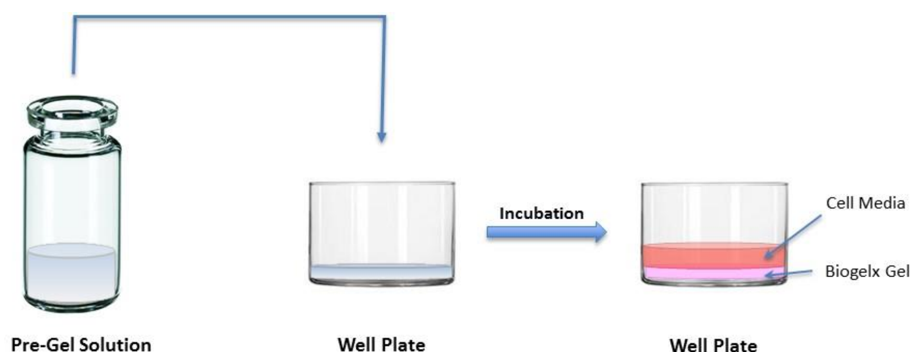
Prior to use, place the cell media and Pre-Gel solution in an incubator or water bath for 30 minutes until the solution(s) reaches 37 °C.

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

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 bath sonicator for 10 seconds.

2D Cell Culture Method (Well Format)

Pipette the Pre-Gel solution into the bottom of the well plate. Volumes of Pre-Gel solution required are shown in **Table 2** 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 2**.

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 2** for cell culture media volumes). 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** before adding cells. During this time, the media will diffuse through the Pre-Gel promoting gelation.

Table 2: 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

Centrifuge trypsinised cells and re-suspend the cell pellet with cell culture media at an appropriate concentration of cells in a final volume. (typically **4 – 16 x 10^4 cells/cm^2** final concentration for most cell types).

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

After the appropriate incubation period, **GENTLY** remove the cell media from top of the Gel and replace with the prepared cell suspension. Cell suspension volumes required are shown in **Table 3** for each plate size used.

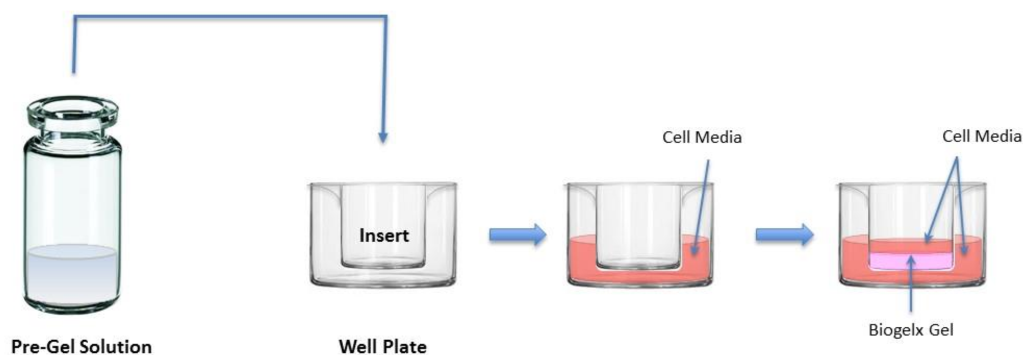
Table 3: Volumes of Cell Suspension required for well plate sizes using 2D cell culture in well format.

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 then every second day after that.

2D Cell Culture Method (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 4** 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, media outside of each insert and Pre-Gel solution in the cell incubator at 37 °C with a humidified atmosphere of 5 % CO_2 for **15 minutes**.

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

To promote gelation, **GENTLY** pipette cell media dropwise onto the centre of the Gel (Refer to **Table 4** 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.

Table 4: 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

Centrifuge the trypsinised cells and re-suspend the cell pellet with cell media, to an appropriate concentration of cells in a final volume (typically **4 – 16 x 10^4 cells/cm^2** final concentration for most cell types).

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 making sure the pipette tip does not touch the surface of the gel whilst doing so. **GENTLY** pipette the prepared cell suspension onto the top of the gel in each insert. Replace the old cell media outside the insert with fresh media. Volumes are shown in **Table 5** for each well plate size.

Table 5: 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	Volume of Cell Culture Media (µL) added outside the insert
24 Well	100	1000
12 Well	300	1400
6 Well	1000	2000

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

3D CELL CULTURE METHOD

Pre-Gel and Cell Preparation

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

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

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 bath sonicator for 10 seconds.

Meanwhile, trypsinise cells and centrifuge so as to obtain a cell pellet. Determine the cell density.

Remove the required volume of cells to a sterile tube to give a final cell density of **0.5 – 1 x 10^6 cells/mL** in Pre-Gel solution.

Centrifuge the cell-media mix for 5 minutes at 1500 rpm to again obtain a cell pellet and remove media, leaving a maximum of 10 % media in which to re-suspend the cell pellet. Disturb the pellet through heavy shaking to allow breaking up of the cell mass. To this, add the required volume of Pre-Gel solution (**0.5 – 1 x 10^6 cells/mL** of Pre-Gel solution).

Mix by **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.

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

3D Cell Culture Method (Well Format)

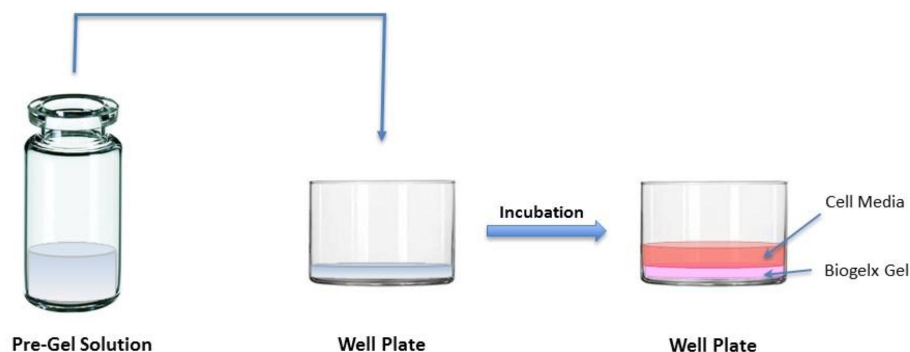
GENTLY pipette the prepared Pre-Gel/cell solution into the bottom of the well plate required. Volumes required for each plate size used are shown in **Table 6**. **Refer to diagram below for guidance.**

Table 6: Volumes of Pre-Gel/cell solution 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)
96 Well	100
48 Well	200
24 Well	500
12 Well	800
6 Well	2500

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 **15 minutes**.

NOTE: At this stage, gelation has not occurred.



To promote complete gelation, **GENTLY** add media dropwise onto the centre of the Gel (Refer to **Table 7** for cell culture media volumes). The Pre-Gel and media should form two distinct layers (**depicted above**). Incubate the prepared well plates at 37 °C with a humidified atmosphere of 5 % CO_2.

Replace the media on the surface of the gel after **2 hours** with fresh media. 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.

Table 7: Volumes of Cell Culture Media required for well plate sizes using 2D cell culture in well format.

Culture Plate Size	Volume of Cell Culture Media added to the Gel surface (µL)
96 Well	150
48 Well	250
24 Well	600
12 Well	1000
6 Well	3000

Incubate the prepared well plates at 37 °C with a humidified atmosphere of 5 % CO_2. After **24 hours** of incubation, replace the media with fresh media.

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

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) and incubate at 37 °C with a humidified atmosphere of 5 % CO_2 for **15 minutes**.

NOTE: At this stage, gelation has not occurred.

Volumes for Pre-Gel/cell solution and media for each culture plate size are shown in **Table 8**.

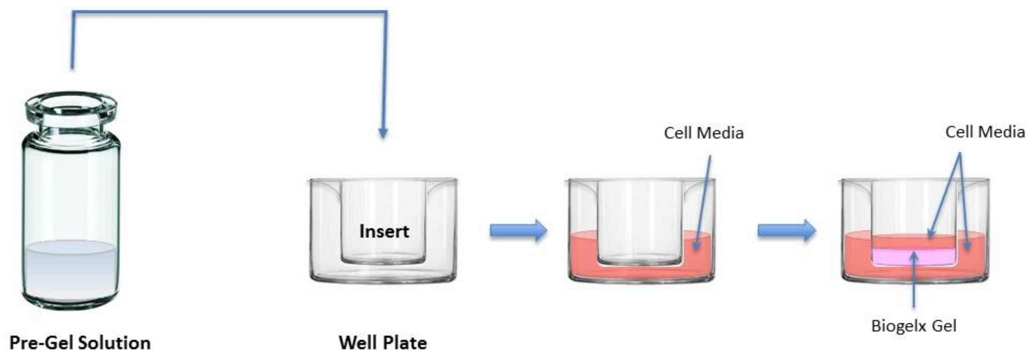


Table 8: Volumes of Pre-Gel/cell solution and Cell Culture Media required for performing 3D cell culture with inserts.

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

After this period, add media **GENTLY** to the surface of the Gel (Refer to **Table 9** for cell culture media volumes) and continue incubation.

Replace the media surrounding the insert (**Table 8**) and on the surface of the gel (**Table 9**) with fresh media after **2 hours**. 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.

Table 9: Volumes of Cell Culture Media required for 3D cell culture with inserts.

Culture Plate Size	Volume of Cell Culture Media added to the gel surface (µL)
24 Well	100
12 Well	300
6 Well	1000

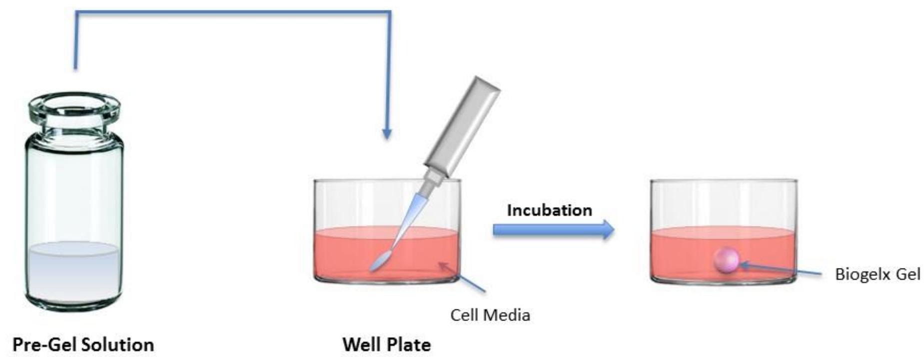
Incubate the prepared well plates at 37 °C with a humidified atmosphere of 5 % CO_2. After **24 hours** of incubation, replace media with fresh media (surface of the Gel and outside the inserts).

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

3D Cell Culture Method (Sphere Format)

Add cell media to each of the desired wells in the required well plate (**Table 10** 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 until gel is fully formed.

Table 10: 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

After **24 hours** of incubation, replace the media in the well with fresh media. Care should be taken when removing the media, to avoid direct contact with the Gel.

Replace the media with fresh media every 48 hours thereafter.

Addition of Growth Factors and Proteins

If using the 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 tissue culture media to obtain the final working concentration.

Prepare Gel following the required Cell Culture Method:

For **2D well plate and insert method**. 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 cell culture protocol method above.

NOTE: At this stage, gelation has not occurred.

To promote gelation, **GENTLY** pipette growth factor/protein and cell media dropwise onto the centre of the Gel (Refer to **Table 2 - 5** for volumes). The Pre-Gel and media should form two distinct layers.

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.

Proceed as described in the Cell Culture Protocol.

For **3D well plate and insert method**. Prepare the Pre-Gel and growth factor/protein with cell media to required volume and concentration. Add to cell pellet as described in Cell Culture Protocol and mix by gently pipetting up and down to allow even distribution of cells in the Pre-Gel solution. Ensure the pipette tip does not leave the solution when mixing as this can introduce air bubbles into the gel structure.

Proceed as described in the Cell Culture Protocol.

If using Biogelx Powder

If suitable, growth factor/protein can be weighed out and directly added to the Biogelx Powder prior to reconstitution.

If additional growth factor/protein is required to be prepared separately and is already in solution this can then be used to reconstitute the Biogelx Powder.

Proceed as described in the Cell Culture Protocol.

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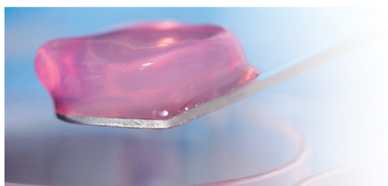

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