

## **Gelacell™ - Gelatin Inserts for 6/12/24 well plate**

*Bringing cell culture to the next dimension*

### **Product Description**

Gelacell™ is a unique, 3D microfibrinous scaffold specifically engineered for advanced in vitro 3D cell culture and tissue engineering applications. Designed as a non-woven, highly porous scaffold, Gelacell™ offers exceptional biocompatibility and non-toxicity across a variety of cell types. Our specially designed scaffold “inserts” offer cell culture conditions for short-to-medium durations. Perfectly tailored for the cultivation of complex systems like cell microenvironments, tissue regeneration, and organoids, Gelacell™ also makes a robust platform for drug screening and other cell culture investigations. The optimized design of the scaffold ensures thermal, chemical, and mechanical stability, while also providing substantial swelling capabilities and porosity, allowing efficient nutrient diffusion, and thereby preventing cellular waste build up.



Figure 1. Gelacell™ inserts in different sizes for 6/12/24 well plates (left to right).

### **Product Features**

- 3D architecture with a substantial available surface area.
- Minimal modifications required for 2D to 3D culture transition.
- Stable mechanical properties.
- Scaffold inserts compatible with 6, 12, 24 well plates.
- Flexibility of the scaffolds facilitates easy handleability.
- Porous structure promotes cell migration and efficient diffusion of nutrients, solutes, and gases.
- Compatibility with a variety of cell lines and culture conditions.
- Biocompatible polymers, diverse and widely accepted in cellular applications.
- Sterilized by Gamma irradiation and will remain sterile until the pack is opened.
- Storage at room temperature.

## Product Datasheet

Product information	Gelacell™
Product code	GCO601RN-IN06-B, GCO601RN-IN12-B, GCO601RN-IN24-B
Polymer	Gelatin (pharmaceutical grade, porcine derived)
Appearance (dry)	Off-white
Appearance (swelled in water/PBS)	Off-white (Translucent)
Fiber orientation <sup>1</sup>	Random
Mean fiber diameter <sup>1</sup>	0.8 - 2.6 μm
Thickness	550 - 750 μm
Area density	25 - 35 g/m <sup>2</sup>
Porosity <sup>2</sup>	94% - 99%
Wettability <sup>3</sup>	Hydrophilic
Swelling in PBS (pH 7.4 at 37°C) <sup>4</sup>	1000% - 1600% <small>The material was kept soaked for 24 hours in PBS at 37°C</small>
Degradation (in PBS at 37°C) <sup>4</sup>	25% - 60%
pH (of PBS solution after 24 hours) <sup>4</sup>	6.9 - 7.5
%Elongation <sup>5</sup>	2.0% - 5.0%
Elastic modulus <sup>5</sup>	1600 - 3000 kPa
Ultimate tensile strength <sup>5</sup>	40 - 80 kPa

- Information is derived from SEM images.
- Porosity is determined by comparing the apparent densities of scaffolds to the bulk densities of Gelatin.
- Wettability is determined by visual observation when water/PBS was added dropwise on top of the scaffold.
- Degradation and swelling tested in PBS solution. Swelling evaluates solution uptake, while degradation is tracked as weight reduction over time with bi-daily PBS renewal.
- Tensile strength measured using a specific in-house protocol, conducted in dry conditions.

## Morphology of Gelatin scaffold

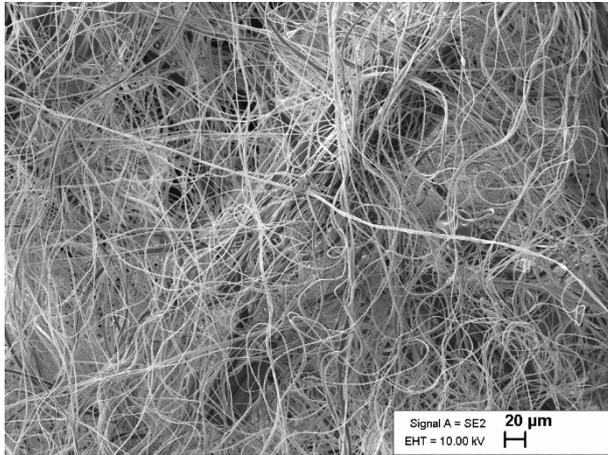


Figure 2. SEM image showing fiber morphology and porosity.

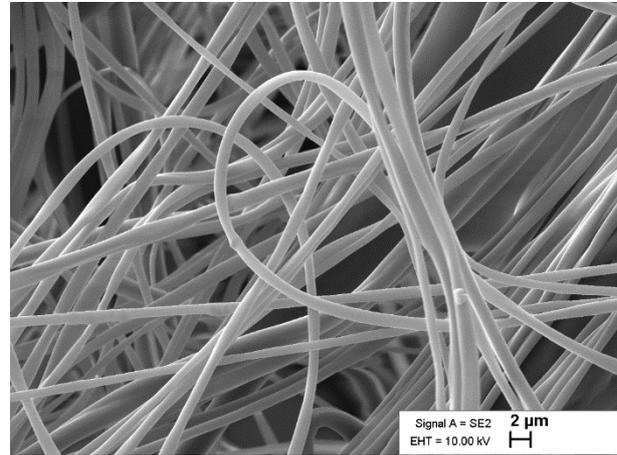


Figure 3. SEM image showing fiber diameter and distribution.

The morphology of fibrous scaffolds plays a pivotal role in its interaction with eukaryotic cells derived from humans and animals. The unique structure of microfibers shown in Figure 2 and Figure 3, mimicking the extracellular matrix, offers a high surface area, and interconnected porous network. This morphology provides an advantageous environment for cell adhesion, proliferation, and differentiation.

## Biocompatibility of Gelatin Scaffold

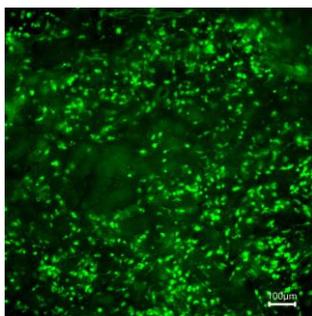


Figure 4. HeLa cells after day 1.

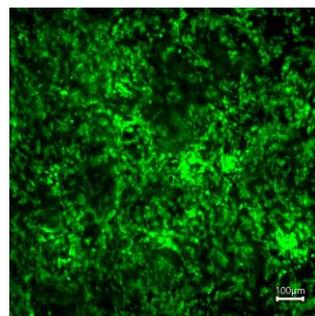


Figure 5. HeLa cells after day 3.

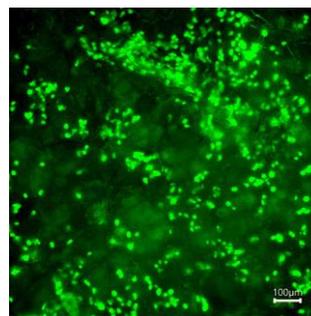


Figure 6. Huh7 cells after day 1.

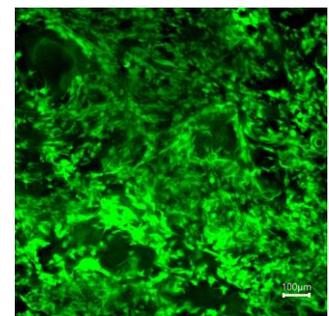


Figure 7. Huh7 cells after day 3.

The scaffolds exhibit promising biocompatibility with various cell types, including HELA, HEK293, HUH7, HEPG2, U2OS, BHK21, and C2C12. Their biomimetic design and specialized polymer composition enhance cell-material interactions, supporting key processes like cellular attachment and spreading - crucial for cell growth and function. HELA and HUH7 cell lines show adherence, proliferation, and infiltration within the scaffold structure (as depicted in Figure 5 to Figure 8). This compatibility is vital across diverse tissue engineering applications, enabling these cell lines to thrive within the scaffold's microenvironment.

## Standard Operating Procedure

### Gelacell unpacking and sterilizing

1. Following the aseptic conditions, put the Gelacell™ package under a sterile laminar flow hood. Carefully cut the boundaries of the sterile pouch; remove the inserts using sterile tweezers/forceps (see Figure 9) and place each insert per well in a multiwell plate (i.e. 6, 12, and 24) according to their sizes.
2. The production of Gelacell takes place in clean room (ISO Class 7) conditions and the products are sterilized by Gamma irradiation. UV sterilization for 20 minutes is advisable to retain sterility of the product after placing the inserts in a multiwell plate.

Note: The scaffolds are expected to remain unchanged under standard UV-C radiation dosage and standard gamma radiation dosage (15-25 kGy). Higher irradiation doses should be used with caution.

### Cell seeding and culture

1. Before cell seeding, rinse the Gelatin scaffold once with 70% ethanol (see table below), followed by a rinse with PBS solution three times.
2. Pre-swell the scaffold with your preferred cell culture media (with/without serum). Add culture media (see table below) onto the scaffold, incubate for 30 mins to 1 hour, and aspirate the spent media.
3. After pre-swelling, allow the scaffold to partially dry in a laminar hood by keeping the lid open for 20 minutes. It helps in absorbing the cell seeding volume and spreading the cells uniformly.
4. Dispense your desired concentration of cell suspension with complete media onto the scaffold. The range of cell seeding density is given in table below as a recommendation, but the exact figure will depend on the cell type and the planned duration of cell culture.
5. Place the well in a CO<sub>2</sub>-incubator at 37°C for 30 mins to 2 hours to allow initial cell adhesion. Afterward, gently fill the well with medium (see table below) from the sides of the well without dislodging the cells that have already adhered to the scaffold. Return the scaffolds to the incubator for culturing cells.
6. It is recommended to exchange media roughly every 24 hours to 48 hours; however, this may vary depending on the cell line, media, and cell density. Aspirate the waste media from the sides of the well and carefully add fresh media (see table below) on top of the scaffold without dislodging the adhered cells. Continue media exchange throughout the cell culture period.

**Note:** The partial drying of scaffold is a crucial step to retain most of the cells during seeding and controls the moisture content before introducing cells. The rate and efficiency of cell attachment and detachment can be affected by temperature, pH, nutrient exchange, the concentration of cells, enzymatic degradation, and cell staining. The above guidelines are the best-known practices based on the tests carried out on the scaffolds.

### Suggested media volume and cell seeding density

Gelacell™ Inserts	Ethanol volume/insert min - max	PBS volume/insert	Pre-swell media volume/insert min - max	Cell seeding volume/insert min - max	Cell seeding density/insert min - max	Cell Culture Media Volume/insert
GC0601RN-INO6-A	800 µl - 1000 µl	5000 µl	1000 µl - 1500 µl	300 µl - 400 µl	$10 \times 10^5$ - $10 \times 10^6$	5000 µl
GC0601RN-IN12-A	400 µl - 600 µl	2000 µl	600 µl - 1000 µl	80 µl - 150 µl	$4 \times 10^5$ - $6 \times 10^6$	2000 µl
GC0601RN-IN24-A	200 µl - 400 µl	1000 µl	200 µl - 300 µl	10 µl - 70 µl	$0.1 \times 10^5$ - $0.8 \times 10^6$	1000 µl

### Cell culture analysis

1. **[Optional] Cell fixation:** Rinse the scaffolds with PBS and use 3.7% - 4% formaldehyde or paraformaldehyde for cell fixation. Leave the scaffolds in the solutions for 15 mins at room temperature or in a CO2-incubator.
2. **For cell viability:** Use assays such as MTS, MTT, CCK-8, etc. for determining the cell activity on the scaffolds. Follow the standard protocols suggested by the manufacturer. The recommended volumes of assays are given in the Table below.
3. **For cell staining and imaging:** Utilize stains such as Calcein, FDA, Phalloidin-conjugates, DAPI, or propidium iodide individually or combined to enhance contrast during imaging. Transferring the inserts to a glass slide can significantly improve cell visualization. Refer to the recommended staining volumes provided in the table below.
4. **Handling:** Employ fine-point tweezers, either straight or angled, to grip the discs (refer to Figure 8). Position the discs facing the scaffolds on a glass slide that contains one or two drops of mounting media. This step is crucial to secure the discs in place. (Figure 8).
5. **Imaging:** Evaluate the scaffolds under a fluorescence or confocal microscope for imaging and visualization.

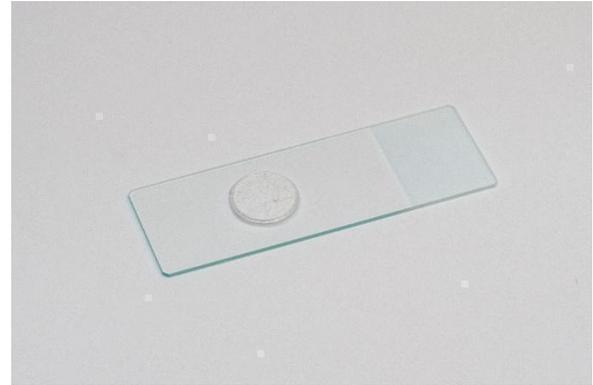


Figure 8. Handling and fixing the Gelacell inserts using mounting media for imaging.

**Note:** The method of cell/tissue culture analysis on the scaffold may vary based on the tests and applications. The above guidelines are based on the tests conducted on the scaffolds. However, users are advised to extend the scope of analysis as per their requirements.

**Suggested volumes of assays and staining solutions**

Gelacell™ Inserts	Assay volume/insert	Staining volume/insert
GCO601RN-IN06-A	1500 µl - 2000 µl	1500 µl
GCO601RN-IN12-A	600 µl - 800 µl	600 µl
GCO601RN-IN24-A	350 µl - 500 µl	350 µl

**Important Information:**

- The scaffold is compatible with both serum and serum-free cultures.
- The inserts are highly flexible (in some cases pliable) therefore, carefully grip the edges of the dry scaffolds when holding or transferring in the wells to prevent flipping and folding of inserts.
- The Gelatin scaffolds after swelling become softer due to their high absorption capacity. It is recommended to gently grip the swelled scaffolds for transferring to glass slides and spreading. Also, avoid forceful pipetting during media aspiration and changing.
- Avoid scratching the scaffold inserts with pipette tips. When pipetting solutions from or into the well, place the tip at the well's periphery to avoid contact with the scaffold.
- Specificity and autofluorescence can cause issues with contrast, but choosing the optimal stain as well as tuning and adjusting the object and intensity of the fluorescence will assist in reaching the desired result.

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- The product maintains sterility until the package is opened, with a shelf life of 24 months from the dispatch date. Both sterility and shelf life are preserved when the product is stored according to the specifications outlined in the Safety Data Sheet.