

## Gelacell™ 3D Microfibrous Scaffold

*Bringing cell culture to the next dimension*

### Product Description

Gelacell™ is a unique, 3D microfibrous 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. This variant of Gelacell™ is specially designed for medium-to-long term cell culture durations. The scaffolds are fixed in a cell crown that act as a Boyden chamber for facilitating studies related to air-liquid interface culture, co-culture, complex tissue models, etc. 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™ scaffolds fixed in a cell crown in 24 well plates.

### Product Features

- 3D architecture with a substantial available surface area.
- Minimal modifications required for transitioning from 2D to 3D culture.
- Stable mechanical properties.
- Scaffold fixed in a cell crown acting as Boyden chamber for complex culture.
- Flexibility of 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.
- Polymeric scaffolds: biocompatibility, diversity, and widely accepted in cellular applications.
- Sterilized by Gamma irradiation and remains sterile until the pack is opened.
- Storage at room temperature.

## Product Datasheet

Product information	Gelacell™
Product code	GC0601RN-CC06-B, GC0601RN-CC12-B, GC0601RN-CC24-B
Polymer	Gelatin (pharmaceutical grade, porcine derived)
Appearance (dry)	White
Appearance (swelled in water/PBS)	White (Translucent)
Fiber orientation <sup>1</sup>	Random
Fiber diameter <sup>1</sup>	1.83 $\mu\text{m}$ $\pm$ 0.87 $\mu\text{m}$
Thickness	0.641 mm $\pm$ 0.016 mm
Area density	28 g/m <sup>2</sup> $\pm$ 1 g/m <sup>2</sup>
Porosity <sup>2</sup>	$\cong$ 87%
Wettability <sup>3</sup>	Hydrophilic
Swelling in PBS (pH 7.4 at 37°C) <sup>4</sup>	$\cong$ 1240% The material was kept soaked for 24 hours in PBS at 37°C
Degradation (in PBS at 37°C) <sup>4</sup>	$\cong$ 54% Scaffold may disintegrate in 28 days
pH (of PBS solution after 24 hours) <sup>4</sup>	7.4 – 7.5 without observable changes
%Elongation <sup>5</sup>	3% $\pm$ 0.27%
Modulus <sup>5</sup>	2127 kPa $\pm$ 182 kPa
Ultimate stress <sup>5</sup>	153 kPa $\pm$ 2.3 kPa

### Note:

1. This information is derived from SEM images.
2. Porosity is determined by using the volumes of scaffolds and Gelatin.
3. Wettability is determined by visual observation when water/PBS was added dropwise on top of the scaffolds.
4. The degradation and swelling tests were performed gravimetrically in PBS solution. Swelling measures the uptake of solution and degradation indicates the decrease in weight over time while PBS was changed after every two days interval.
5. The tensile measurements were performed according to an in-house protocol and in a dry state.

## 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 and remove the well plate.
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 upon unpacking.

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 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. Dispense your desired concentration of cell culture 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.
4. Place the well in a CO<sub>2</sub>-incubator at 37°C for 30 mins to 2 hours to allow initial cell adhesion. Afterwards, gently fill the well with the medium (see table below) without dislodging the cells that have already adhered to the scaffold. Incubate for 24 hours to ensure complete cell adhesion to the scaffold.
5. [Optional] Evaluate cell adhesion under a microscope after incubation and increase the media volume (see table below) to provide an appropriate medium for cell culture.
6. It is recommended to exchange media roughly every 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.

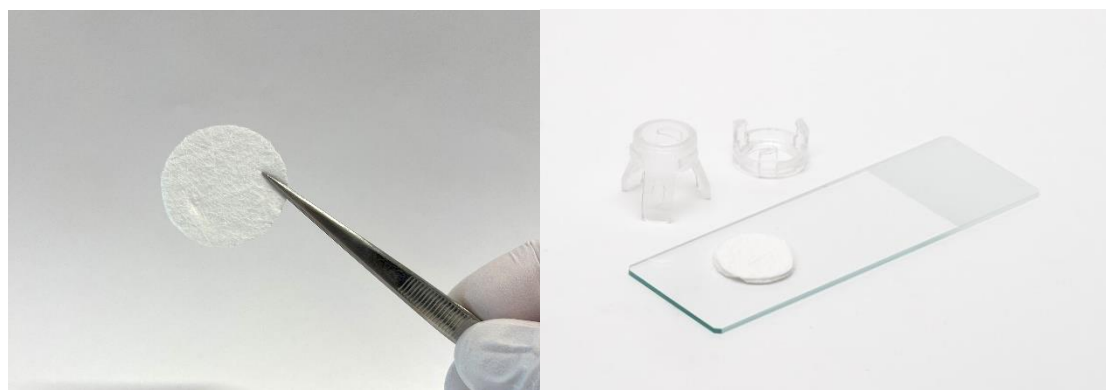
Note : The rate and efficiency of cell attachment and detachment can be affected by temperature, pH, nutrient exchange, the concentration of cells, enzymatic degradation, and cell staining. The above guideline is the best known practices based on the tests carried out on the scaffolds.

#### Suggested media volume and cell seeding density

Scaffold in cell crown	Ethanol volume/insert	PBS volume/insert	Pre-swell volume/insert	Cell seeding volume/insert	Cell seeding density/insert	Media Volume/insert
GC0601RN-CC06-B	800 µl - 1000 µl	5000 µl	1500 µl - 2000 µl	300 µl - 400 µl	10 × 10 <sup>5</sup> - 10 × 10 <sup>6</sup>	5000 µl
GC0601RN-CC12-B	400 µl - 600 µl	2000 µl	900 µl - 1500 µl	80 µl - 150 µl	4 × 10 <sup>5</sup> - 6 × 10 <sup>6</sup>	2000 µl
GC0601RN-CC24-B	200 µl - 400 µl	1000 µl	350 µl - 500 µl	10 µl - 70 µl	0.1 × 10 <sup>5</sup> - 0.8 × 10 <sup>6</sup>	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 CO<sub>2</sub>-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.
3. **For cell staining and imaging:** Use stains (e.g., calcein, FDA, phalloidin-conjugates, DAPI, propidium iodide) alone or in combination to achieve better contrast when imaged. Open the cell crown and transfer the scaffolds on a glass slide for visualizing cells.
4. **Handling:** Use fine point tweezers/forceps (straight or angled) to hold the scaffolds (see Figure 2), and place it on a glass slide. Add one or two drops of mounting media to fix the scaffolds and cover it with cover slip (Figure 2).
5. **Imaging:** Evaluate the scaffolds under a fluorescence or confocal microscope for imaging and visualization.



**Figure 2:** Fix the Gelacell discs 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.

### Important Information:

- The scaffold is compatible with both serum and serum-free cultures.
- The scaffolds are highly flexible (in some cases pliable) therefore, carefully grip the edges of the scaffolds when holding or transferring in the wells to prevent flipping the scaffolds. Avoid forceful pipetting.
- 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 while using micropipettes. 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.
- Sterility and performance properties are maintained when stored at room temperature, with a shelf life of two years from the date of manufacture.

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