Gelacell™ - PLLA 6/12 well plate with cell crowns

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™ 6/12 well plates with cell crowns (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 fixed in a cell crown acting as Boyden chamber for complex culture.
- 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.

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Product Datasheet

Product information	Gelacell™
Product code	GC0805RN-CC06-B, GC0805RN-CC12-B
Polymer	Poly-L-lactide (PLLA) (Pharmaceutical grade)
Appearance (dry)	White
Appearance (swelled in water/PBS)	White (Translucent)
Fiber orientation[1]	Random
Mean fiber diameter[1]	0.4 – 1.4 µm
Thickness	140 - 230 µm
Area density	3.0 – 9.0 g/m^2
Porosity[2]	95% - 99%
Wettability[3]	Hydrophilic The material uptakes water, and compounds that dissolve in water due to the porosity and capillary forces wherever the material is soaked in a solution and incubated.
Absorptivity in PBS (pH 7.4 at 37°C)[4]	600% - 1200% The material was kept soaked for 24 hours in PBS at 37°C
Degradation (in PBS at 37°C)[4]	Up to 20%
pH (of PBS solution after 24 hours)[4]	7.3 – 7.5 without observable changes
%Elongation[5]	20% - 45%
Elastic modulus[5]	400 – 1300 kPa
Ultimate tensile strength[5]	80 – 220 kPa

1. Information is derived from SEM images.
2. Porosity is determined by comparing the apparent densities of scaffolds to the bulk densities PLLA.
3. Wettability is determined by visual observation when water/PBS was added dropwise on top of the scaffolds after washing the scaffolds with 70% ethanol. Ethanol washing is highly recommended as PLLA is inherently hydrophobic in nature.
4. Degradation and absorptivity tested in PBS solution. Absorptivity evaluates solution uptake, while degradation is tracked as weight reduction over time with bi-daily PBS renewal.
5. Tensile strength measured using a specific in-house protocol, conducted in dry conditions.

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Morphology of PLLA scaffold

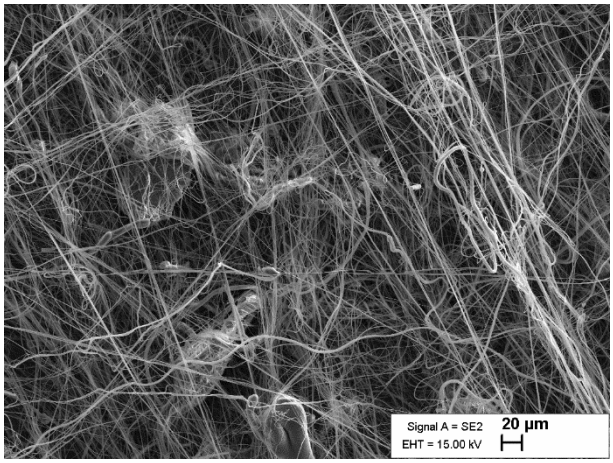

Figure 2. SEM image showing fiber morphology and porosity.

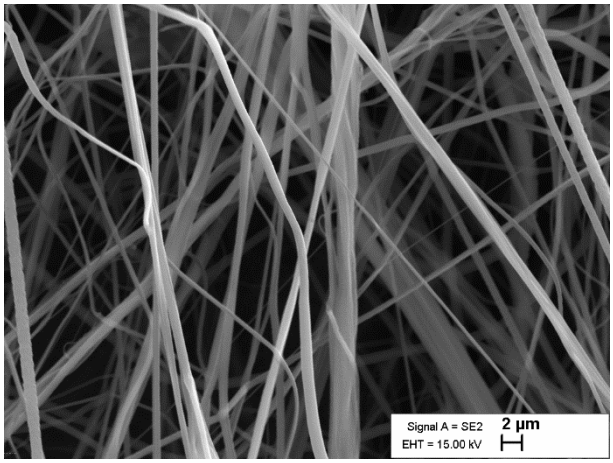

Figure 3. SEM image showing fiber diameter and distribution.

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 PLLA Scaffold

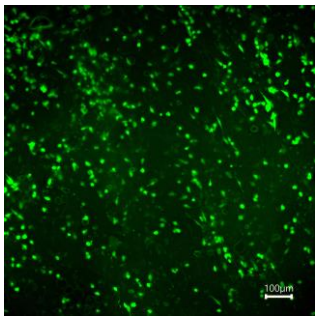

Figure 4. Hela cells after day 1.

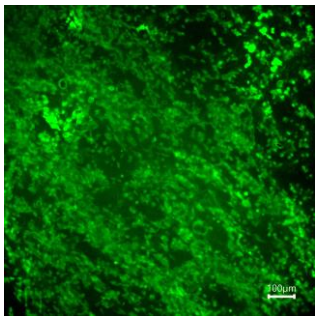

Figure 5. Hela cells after day 3.

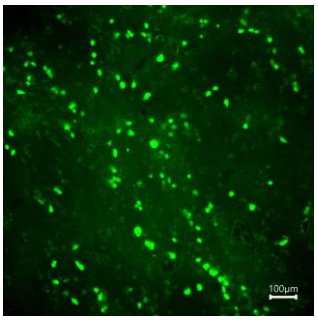

Figure 6. Huh7 cells after day 1.

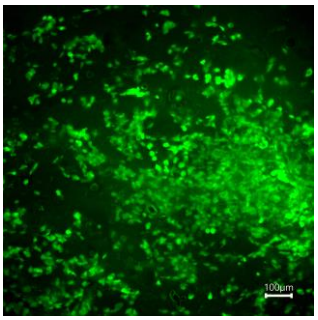

Figure 7. Huh7 cells after day 3.

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 4 to Figure 7). This compatibility is vital across diverse tissue engineering applications, enabling these cell lines to thrive within the scaffold's microenvironment.

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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 PLLA 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 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.
5. Place the well in a CO2-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™ well plate with cell crowns	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
GC0805RN-CC06-B	800 µl - 1000 µl	5000 µl	1500 µl - 2000 µl	300 µl - 400 µl	10×10^5 - 10×10^6	5000 µl
GC0805RN-CC12-B	400 µl - 600 µl	2000 µl	900 µl - 1500 µl	80 µl - 150 µl	4×10^5 - 6×10^6	2000 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:** Unclip the cell crown by rotating the bottom part (small part). Use fine point tweezers/forceps (straight or angled) to hold the scaffolds (see Figure 8) and place it on a glass slide. Add one or two drops of mounting media to fix the inserts and cover it with cover slip (Figure 8).
5. **Imaging:** Evaluate the scaffolds under a fluorescence or confocal microscope for imaging and visualization.

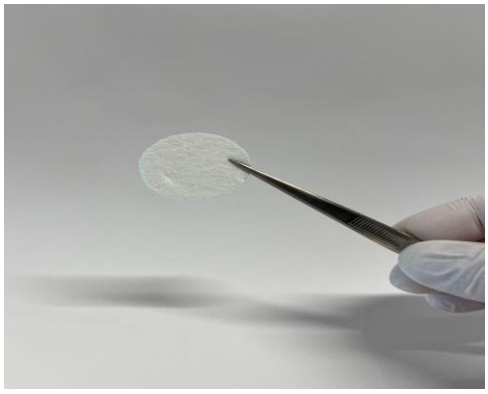

Figure 8. Fix the Gelacell discs using mounting media for imaging.

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.

Suggested volumes of assays and staining solutions

Gelacell™ well plate with cell crowns	Assay volume/insert	Staining volume/insert
GC0805RN-CC06-B	2000µl - 2500 µl	2000 µl
GC0805RN-CC12-B	800 µl - 1000 µl	800 µl

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.
- 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.
- 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.

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