Porocell-3D
Kit for 12 well plate

NOT intended for human use - For research use only!

Description and advantages:

Porocell™-3D is a user-friendly 3-D cell culture matrix for soft tissue engineering applications that provides conditions that promotes normal cell morphology and behavior to mimic the natural environment. The Porocell™-3D matrix is an animal free and biocompatible poly-L-lactic acid based polymer scaffold. The hydrophilic matrix is extremely permeable from the top but only slightly permeable from the bottom. This allows to seed cells efficiently on the matrix surface, allows cells to be retained in the matrix and is optimal for nutrient supply. The highly porous architecture is effective for culturing a high density of immortalized and primary cell suspensions: Supports short- and long-term growth and differentiation, promotes cellular proliferation of a variety of cell types, including epithelial cells (e.g., hepatocytes), fibroblasts, cancer cells (breast) of different species. Porocell™-3D exhibits highly inter-connective pore structure that provides superior cell loading capacity and nutrient delivery compared to standard 2D cell culture systems. The cell culture grade coating system improves cell adhesion for certain cell type. The Porocell™-3D matrix is suited for in vitro and in vivo studies.

Applications for Porocell™-3D

Academic and industrial applications: Porocell™-3D is suitable for several cell-based and cell-contact based research and industrial uses: high-throughput screening assays, toxicology, drug development, multicellular assays, cancer and stem cell research as well as tissue and organ engineering. Porocell™-3D can mirror in vivo settings and therefore provides more realistic in vito results.

Product Qualification

The Certificate of Analysis (CofA) with quality control information for each lot number (printed on the label) of Porocell™-TX is available for download on our website at www.hacbiomed.com/certificates/.

Porocell™-3D Cell Culture Assay Set-up

1. Porocell™-3D is delivered in a standard sterile well-plate format that is preloaded with hydrophilic scaffolds. Cells suspension can be loaded directly.
2. Perform all procedures under aseptic conditions, in a laminar flow hood.
3. Remove Porocell™-3D plate from his package.
4. You can use all the wells of each plate at the same time and perform your assay in the provided cell plate. The matrix can be transferred in another recipient.

5. **Transferring unloaded scaffold**
   a) To remove the matrix, use the provided sterile tweezers. Pull carefully from the border of the scaffold. **Do not pull up the Porocell™-3D matrix from its center!**
   b) If you plan use only part of the scaffolds, transfer them to a cell culture plate of the same format or to a culture dish of your choice.
   c) If you wish to incubate the scaffolds in higher amount of medium, transfer them to a culture dish of your choice or.

6. **Note:** Leftover scaffolds can be stored at 5-15 °C if packaged in the original pouch and sealed.

7. Prepare cells for use in 3D cell culture as per standard procedures. Seeding density varies with cell type, but a typical range is shown in the table below. Set the cell concentration such that the proper cell density is contained in the recommended volume. Do not exceed this volume when inoculating in order not to seed cells on the plate bottom instead.

Cell number	Inoculation volume [μl]	Cell concentration [cells/ml]
0.5×10^6	300	1.7×10^6
7.0×10^6	300	23.3×10^6

8. **Note:** Optimizing concentration for inoculation and incubation conditions may improve performance.

9. Seed cells by carefully and slowly pipetting the suspension in the middle of Porocell™-3D scaffold.

10. Allow the plate to rest for 3 min under the laminar flow hood to let cell suspension infiltrate spontaneously.

11. **Addition of medium after inoculation**

    a) Keep the tip at an angle against the wall and do not allow the pipette tip to contact the scaffold when adding medium.
    b) Don´t create stir when adding the medium.
    c) Pipette slowly and carefully the appropriate medium according to table xxx or to your assay design.

12. Incubate the construct in humidified incubator.

13. Cultures inoculated at lower densities may need media replacement when the media turns yellow (The re-feeding period should not exceed 5 days). For high density cultures we recommend to replenish the medium daily by gently removing spent medium per well and adding an equivalent amount of fresh medium as described in step 11.

14. After desired experimental incubation the Porocell™-3D matrix can easily be removed gently and carefully using the provided sterile tweezers and examined or used for downstream applications. Pull carefully from the border of the scaffold. **Do not pull the Porocell™-3D matrix from its center!**

Recomendations to carry out downstream assays:

- For cell viability and proliferation assay, we recommend water soluble system e.g WST cell counting system to avoid extraction step and directly assay.
- Supernatants can be sampled directly for downstream analysis.
- For in –situ staining, embed Porocell™-3D cell construct before sectioning.
- Porcell™-3D cell or neotissue constructs can be frozen at -80°C for short term or in liquid nitrogen using Porofreeze™ storage medium from HAC Biomed for long term storage.