Ossiform
We Print Bone™

Product Data Sheet

Revision 1.0
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Ossiform ApS

P3D Scaffolds

Scaffold sizes:

Ø5 mm
Ø12 mm
Ø20 mm
Ø30 mm

Usage restrictions: For Research Use Only. Not For Use in Diagnostic Procedures. Not for Use in Clinical Studies.

Product description:

P3D Scaffolds are xeno-free bioceramic scaffolds which provide a bone-like niche for *in vitro* and *in vivo* studies such as disease modeling, drug screening and bioengineering. The 3D printed bioceramic scaffolds are developed to resemble native bone with respect to both physical and structural properties, such as high contents of β-tricalcium phosphate and stiffness, as well as macro- and micro-porosities.

The P3D Scaffolds are an easy plug-and-play solution for 3D cell culturing of bone harboring or bone related cell types. The cell culture system is compatible with most common laboratory techniques and does not require major alterations of preexisting protocols and workflows.

Specifications	
Formulation	Beta-tricalcium phosphate Cas Nr 7758-87-4
Use	3D Cell culturing
Operation	Ready-to-use at room temperature
Biocompatibility	Biocompatible, approved for animal studies
Cell harvesting	Use traditional cell harvesting techniques
Storage	Store dry at room temperature
Plate type	Compatible with most common plate types. Ultra-low attachment plates are advised.

P3D Scaffolds provide you with a clinically relevant cell culture system of natural materials and customized structures which allow you to create predictive research models of human physiology and pathology.

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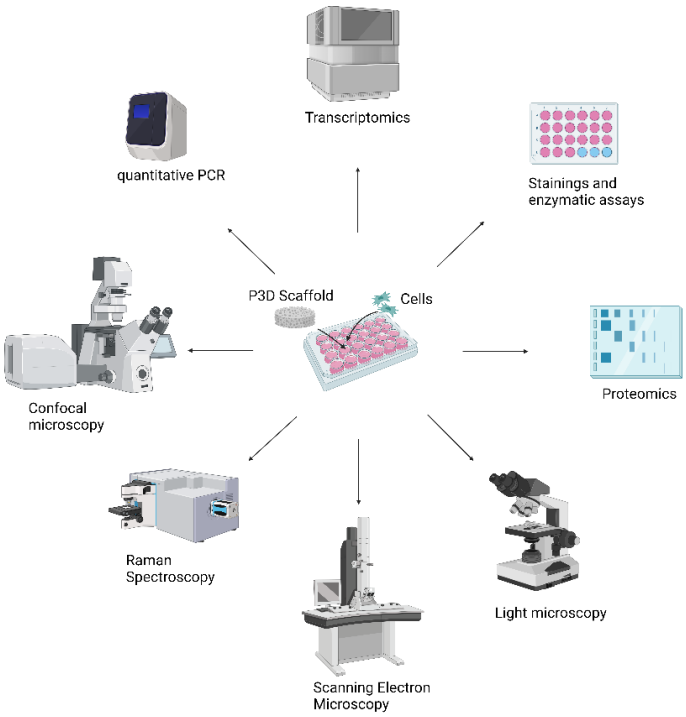


Analyses compatible with P3D Scaffolds

Protocols

Please visit the website www.ossiform.com/ossiform-research-line.aspx to retrieve all protocols, product promotion file, guidelines, and FAQs about the usage of P3D Scaffolds. All video protocols can be found on Ossiform's YouTube channel at www.youtube.com/@ossiform.

Choose the infill type that is best suited for your project

P3D Scaffolds come with two different infill types – grid and gyroid.

Different infill types are recommended depending on the study design and subsequent analyses. If you plan to use P3D Scaffolds for traditional cell culturing and wish to evaluate cell proliferation throughout the incubation period, we suggest that you use P3D Scaffolds with a grid infill as this infill allows for easier cell evaluation by bright-field microscopy.

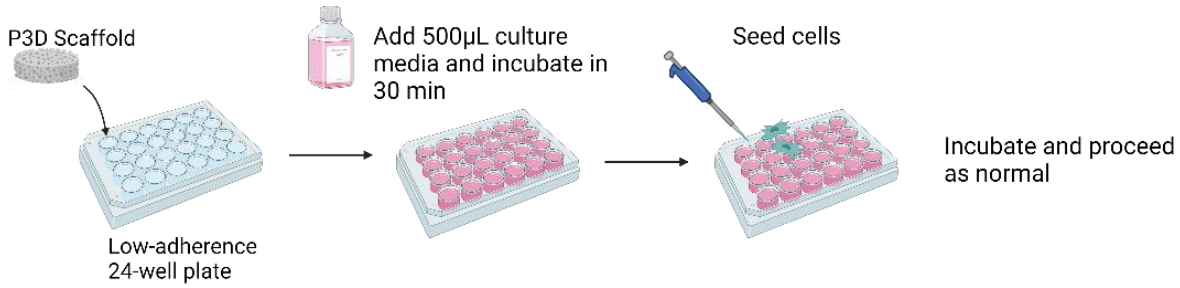
If your study is not dependent on easy bright-field microscopy access, we recommend the gyroid infill. Here you will have a P3D Scaffold with a more organic organization of the macro-porous structures. Bright-field microscopy is still possible to perform on P3D Scaffolds with gyroid infill, it just requires longer time at the microscope per sample.

Are you planning on conducting animal studies using P3D Scaffolds? In that case, we always recommend the gyroid infill for an optimized ingrowth of blood vessels.

If needed, the P3D Scaffolds can be customized in shape and size to accommodate your specific study setup.

3D cell culturing using P3D Scaffolds

This protocol is based on Ø12mm Scaffolds for 24-well plates. The same protocol applies for all other sizes of P3D Scaffolds. Volumes may need adjustment to accommodate other well sizes.



Preparation of the Scaffolds

1. Place one P3D Scaffold in each well of a low-attachment 24-well plate
2. **Optional:** Wash the P3D Scaffolds in 500 uL PBS x3 for one minute to remove any particles.

Note: The material and structure of the P3D Scaffold are not compromised during sterilization or disinfection, but loose particles may occur.

Seeding of cells

1. If not done already, add one sterilized or disinfected P3D Scaffolds to each well of a 24-well plate.
2. Add 500µL culture media to each well and let the scaffold soak. The scaffold must be completely submerged in medium.
3. Incubate the plate in a humidified incubator for at least 30 minutes, at 37°C and 5% CO_2.
4. Add 500uL of the cell suspension to each well by placing the cells on top of the scaffold.
5. Replace the medium and evaluate cell status after 24 and 48 hours.
6. Incubate for a duration suited for your setup.
7. The organoids are now ready for analysis and further experiments.

   Optional: If needed, you may retrieve the seeded cells from the scaffold as described in P3D Scaffold Cell Recovery by Trypsinization protocol available at ossiform.com.

Due to the large surface area, and therefore significantly larger culture area as compared to traditional 2D cell culturing, P3D Scaffolds require a larger seeding density. Please find the recommended seeding densities in the table to the right. Following these recommendations will allow the cells to reach confluency at a similar rate to the one observed in 2D cell culturing.

Seeding densities	
Ø5mm (96 well format)	30.000 cells
Ø12mm (24 well format)	350.000 cells
Ø20mm (12 well format)	500.000 cells
Ø30mm (6 well format)	1.000.000 cells