PROTOCOL

P3D Scaffolds Quick Start
— 3D cell culturing on P3D Scaffolds

Application:

P3D Scaffolds are three-dimensional and biocompatible 3D cell culture and drug screening systems made from 3D printed β-tricalcium phosphate (β-TCP). P3D Scaffolds are available in three standard formats: Ø12 mm, Ø20 mm, and Ø30 mm which are suitable for short-term and longer-term experiments. All formats are suited for demanding cell types as well as co-culturing. If you are unsure which scaffold format to use, feel free to reach out to Particle3D at: sales@particle3d.com.

This protocol provides general guidelines on how to grow 3D cultures on P3D Scaffolds. It is a suggestion for the 3D culturing procedure for a wide range of cells, including but not limited to, stem- and cancer cells, which can be combined with standard recommendations for your specific cell type.

For more protocols for specific research applications and cells, please visit our Resources Platform.

Notes before starting and general advice on material handling

- All handling of the P3D Scaffolds products should be performed using gloves, according to standard aseptic methods.
- The scaffolds are supplied sterile by dry heat sterilization and remains sterile until opened.
Preparing the scaffolds

1. Remove the P3D Scaffolds from the packaging and inspect the scaffolds. Discard any damaged scaffolds.

2. Place one P3D Scaffold in each well of a suitable multi-well plate. We recommend using low-adherence well plates. The choice of well plate depends on the scaffold size you are using.

3. **Optional:** Wash the sterilized P3D Scaffolds in 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.

4. **Optional:** If desired, the P3D Scaffolds can now be coated. For example, it is possible to coat the scaffolds with a number of ECM proteins such as Fibronectin or collagen, using standard protocols supplied by the manufacturer.

*Figure 1: Inspect the P3D Scaffolds and place them in a suitable well plate.*
Preincubation & Seeding

1. Before adding your cells, soak the scaffolds in culture media. The volume of culture medium depends on the product format you are using. Make sure to add enough so that the whole scaffold is completely submerged in medium.

2. Place the plate in a humidified incubator for at least 30 minutes, at 37°C and 5% CO₂.

3. After preincubation, dissociated cells may now be seeded, or spheroids applied manually, into the scaffolds using standard cell seeding protocols.

4. For optimal results, seed the cells in the middle of the well and be careful not to touch the scaffold. Since the P3D Scaffolds provide a much greater surface area than standard 2D culture plates, the seeding densities should be slightly higher than the density used for 2D culture. Initial cell densities of $10^4 – 10^6$ cells/cm² are suggested. The cell density should be chosen based on your cell type and experimental needs.

5. For experiments requiring long incubation times, change media using standard protocols at the normal rates suggested for your cell type. In general, 3D cultures require longer incubation times and more rigorous shaking compared to 2D culture.

Post-processing

Following proliferation, the cells and scaffolds can be used for a wide variety of post-processing applications, including normal protocols for immunochemistry, microscopy, and in-vitro functional studies.
## Recommended seeding densities

<table>
<thead>
<tr>
<th>Plate size</th>
<th>Min. cell density</th>
<th>Max cell density</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 well</td>
<td>9 5000 cells/well</td>
<td>9 500 000 cells/well</td>
</tr>
<tr>
<td>12 well</td>
<td>38 000 cells/well</td>
<td>3 800 000 cells/well</td>
</tr>
<tr>
<td>24 well</td>
<td>19 000 cells/well</td>
<td>1 900 000 cells/well</td>
</tr>
<tr>
<td>48 well</td>
<td>9500 cells/well</td>
<td>950 000 cells/well</td>
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</tbody>
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