Guidelines for Cell Seeding and Proliferation in 3D LifeGel

Cell Seeding Density Recommendations

Optimal cell seeding density in 3D culture—similar to 2D systems—depends on factors such as cell type, intended culture duration, and assay endpoint. The guidelines below offer starting points for 3D cultures maintained for approximately 7–10 days.

For longer 3D culture durations, consider reducing the seeding density to prevent over confluence. Conversely, shorter culture periods may benefit from higher initial densities. Rapidly proliferating cell lines (in 2D or 3D) typically require lower seeding densities, while slower-growing cells may need higher densities to achieve optimal results.

3D Cell Seeding Protocol for 96-Well Plates

For seeding cells into 3D LifeGel in 96-well plates, begin by gently aspirating the equilibration medium from above the gel surface. Carefully dispense **150 µL** of fresh culture medium containing **2000 cells per well** onto the gel. Under typical conditions, cells will continue to grow for **6–7 days** before requiring a medium change. If satisfactory 3D growth is observed, subsequent medium changes should occur every **3–4 days**.

Medium volumes can be adjusted to suit specific experimental needs. For example, reducing the medium volume to **75 µL** per well allows additional space for assay reagents, such as those used in luminescence-based viability assays.

Seeding Density Considerations

While 2000 cells per well is a commonly used starting point, the optimal seeding density may vary depending on cell type, proliferation rate, and experimental objectives. For comparative purposes, consider the following seeding densities:

- **1000 cells/well**: Suitable for fast-growing cells or longer culture durations, helping to avoid early overgrowth and nutrient depletion.
- **2000 cells/well**: Recommended for general use and balanced growth over a standard 7–10 day culture period.
- **3000 cells/well**: Ideal for slow-growing cells or when a shorter culture period is desired, enabling earlier attainment of sufficient cell mass for downstream assays.

Adjusting seeding density enables better control of growth dynamics, nutrient usage, and assay timing, ultimately enhancing the reliability and reproducibility of 3D culture results.

REAL RESEARCH

Real Research S.A.
Jagiellonian Center of Innovation
Prof. M. Bobrzyńskiego 14
30-348 Kraków, PL

The Bradfield Centre
184 Cambridge Science Park
Milton Road,
Cambridge, CB4 0GA, UK

Guidelines for Cell Seeding and Proliferation in 3D LifeGel

384-Well Plates: 500 Cells per Well

Following the removal of the equilibration medium, gently dispense **60 µL** of culture medium containing **500 cells** into each well. Recommendations for cell growth and medium changes are consistent with those for 96-well plates. For assay compatibility, the medium volume can be reduced to approximately **40 µL**, providing space for the addition of detection reagents.

48-Well Plates: 4000 Cells per Well

After aspirating the equilibration medium, add **300 µL** of culture medium containing **4000 cells** into each well. As with other formats, cell growth behavior and medium change intervals should follow the same guidance as for 96-well plates. For assay purposes, medium volume may be reduced to **150 µL**, depending on downstream application needs.