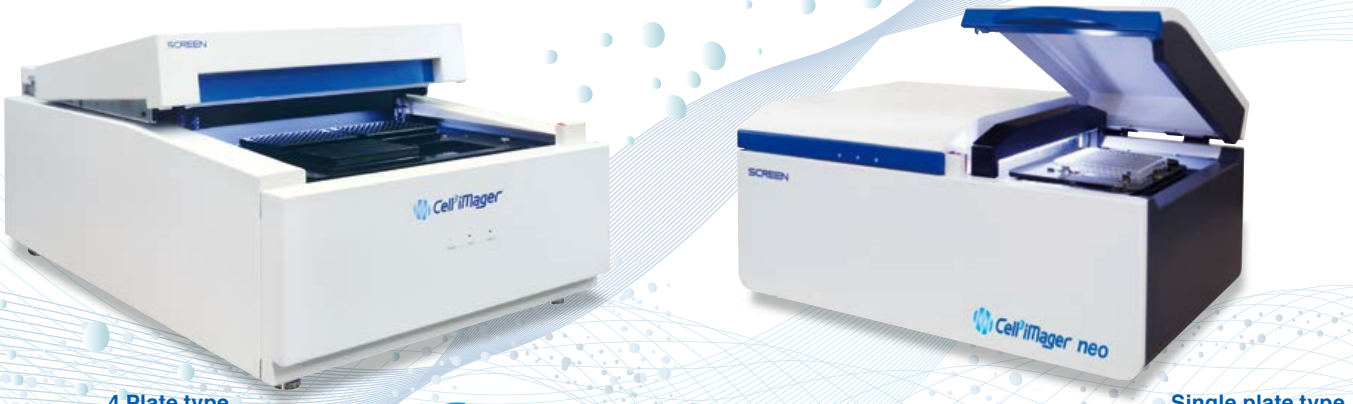
SCREEN

High-Speed 3D Cell Scanner Application Note

Rapid production and size assessment of Embryoid Bodies (EBs)



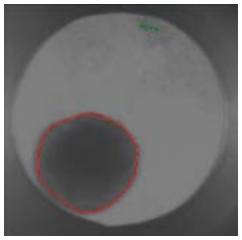
4 Plate type

Single plate type

Cell³iMager

Simplify formation of EBs with GravityPLUS™ hanging drop plates and Cell³iMager analysis

- Produce consistently sized EBs in a 96-well automation-compatible format
- Eliminate suspension culture and tedious manual selection of EBs with simple transfer to culture plates
- Monitor EB growth and differentiation with rapid , label-free imaging



Sizeanalysis of embryoid body at day 5 of culture using the Cell³iMager

A no hassle solution for production of consistently sized EBs

The establishment of 3-dimensional embryoid bodies(EBs) from pluripotent stem cells holds promise as tool for regenerative medicine, in vitro toxicity testing, and as a model for studying embryogenesis.

Large-scale production of EBs for use in such purposes can be accomplished by seeding single ESCs in rotating suspension culture, a process resulting in large numbers of EBs, but limited in its ability to reliably produce EBs of consistent size. Alternatively, more consistently sized EBs can be formed using the hanging drop method, in which single-cell suspensions are seeded and grown on petri dish lids, transferred to suspension culture, then hand-picked for analysis in high-throughput culture plates.

However this tedious, labor-intensive process can also result in size variations resulting from fusion of EBs during the suspension culture phase.

InSphero's patented GravityPLUS™ hanging drop platform provides a simple, automation-compatible solution to the production of consistently sized assay-ready EBs.

The SureDrop™ inlet allows hassle-free formation of EBs in hanging drop by simple top-loading of cells in suspension.

The stable 96-well format increases throughput, reduce waste, and eliminates the need for suspension culture and cumbersome hand-picking of similarly sized EBs. Subsequent analysis with SCREEN Cell³iMager provides label-free monitoring of EB size and morphology over days in culture, ensuring consistency of EB production and as an endpoint for growth and differentiation.

Applications

- Embryotoxicity / teratogenicity screening
- Differentiation pathway analysis
- Quality control

Upgrade your cell-based assays to 3D

Rapid production of Embryoid Bodies

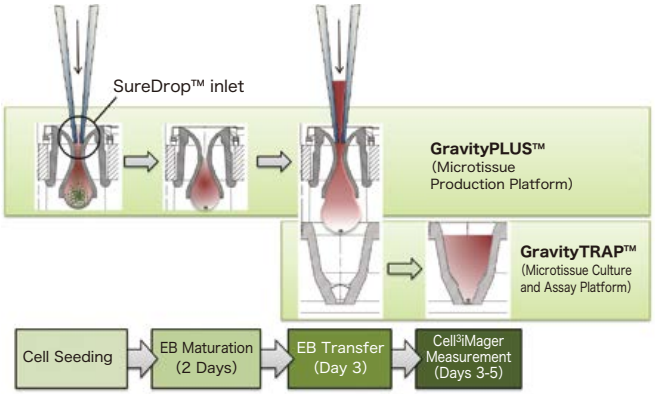
Never flip your lid again!

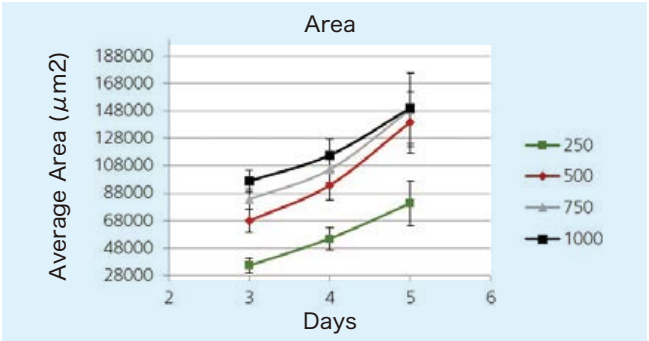
inSphero Biomimetic Microtissue Technology

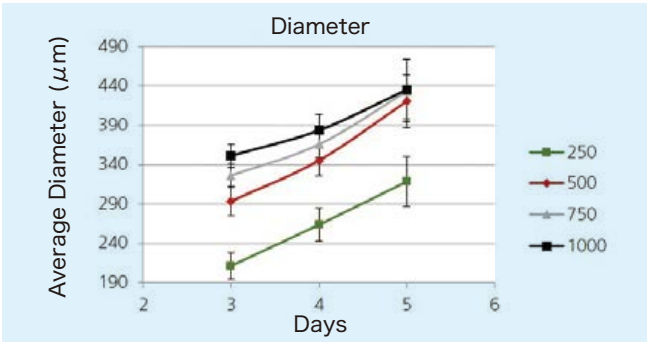
Simplify formation of EBs with GravityPULS™ hanging drop plates

ESD3[D3](ATCC® CRL1934™) were expanded on gelatin coated flasks in serum containing stem cell medium supplemented with LIF. Subsequently, ESD3 cells were trypsinized and harvested to re-seed in the 96-well GraveityPLUS™ hanging drop platform at densities of 250, 500, 750 and 1000 cells/well (24 replicates per density).

Cells were allowed to re-aggregate to form EBs in a serum supplemented cell differentiation medium.

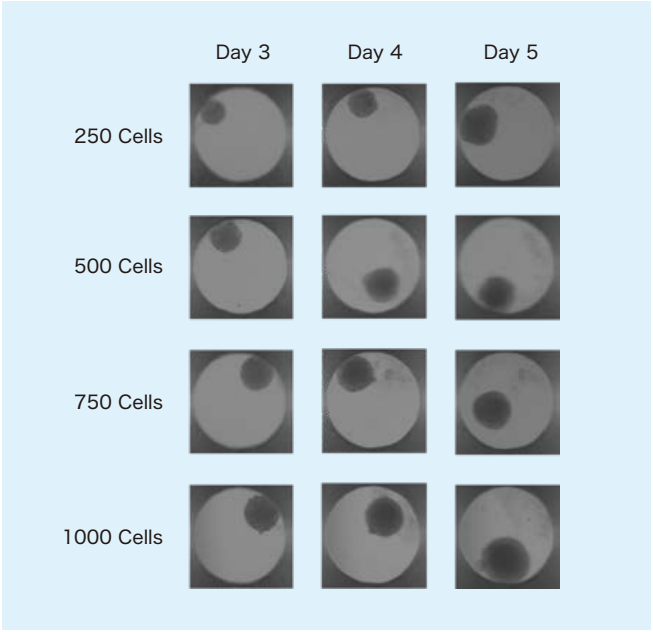






Assessment of EB size in GravityTRAP™ plates using the Cell³iMager

After 3days, developed EBs were transferred into the GravityTRAP™ non-adherent flat bottom plate by addition on fresh culture medium trough the SureDrop™ inlet. EBs were monitored for growth at 3 times points (days 3, 4 and 5) with an optical read-out using the Cell³iMager (SCREEN Holdings Co., Ltd. Japan) at a resolution of 4800 dpi. Captured images were used to calculate average EB area (top), diameter (bottom) and optical density(data not shown) applying an algorithm specific for EB size assessment.



Top: overview of EB formation in the GravityPLUS™ hanging drop platform and subsequent transfer to GravityTRAP™ plates for size analysis on Cell³iMager. Bottom: representative images depicting EBs following transfer and culture in 96 well-GravityTRAP™ plate(day 3 = day of transfer, well diameter = 1mm)

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BCS-102(E)(R1-1) Printed in Japan 06-18 005BB