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Simplifying Progress

Monitor and characterize iPSC culture and differentiation using Advanced Flow Cytometry and Live-Cell Analysis

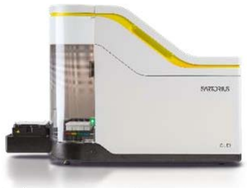
D. Cole*, K. McBain, N. Bevan and T. Dale

BioAnalytics, Sartorius (Essen BioScience), Royston, Hertfordshire, UK;
*Corresponding author: Daryl.Cole@sartorius.com

Introduction

- iPSCs are intrinsically valuable due to their unique characteristics and the control they afford to enable researchers to investigate early stages in cellular development.
- The major benefits of iPSCs are the variety of cell types they can be differentiated into and their capacity for infinite expansion.
- This flexibility provides opportunities for development of cell and tissue models in both 2D and 3D for pharmacological testing, cancer research, organoid modelling of tissues and neurodevelopmental biology.
- iPSCs are increasingly used in translational applications, targeting eventual clinical use via autologous cell therapies and individualized medicine approaches.
- Despite their broad application potential, iPSCs are high maintenance, expensive, and require constant monitoring to ensure they maintain pluripotency, viability, and homogeneity.
- Here we demonstrate how you can evaluate and monitor the pluripotency of iPSCs both in 2D and 3D culture systems by cell surface marker evaluation using the iQue® Advanced Flow Cytometer and morphological analysis using the Incucyte® Live-cell analysis platform.
- Long-term hepatic iPSC differentiation experiments can also be successfully monitored and validated throughout the time-course, providing a streamlined solution for differentiation quality control experiments.
- Maintenance of pluripotent phenotype can be achieved without daily media changes using Sartorius® Research Use Only (RUO) growth factors and cytokines.

Incucyte® & iQue® Systems



iQue® Advanced Flow Cytometer

An advanced flow cytometry platform with a patented sampling method allowing for rapid sample acquisition to deliver fast actionable results. Capable of handling 96 and 384 well plates.

Incucyte® Live-Cell Analysis System

A fully automated phase contrast and multi-color fluorescence system that resides within a standard cell incubator for optimal cell viability. Designed to scan plates and flasks repeatedly over time.

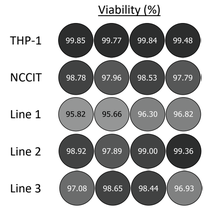
Sartorius Reagents and Consumables

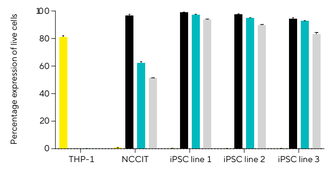
A suite of reagents, kits and protocols for cell health and function screening.

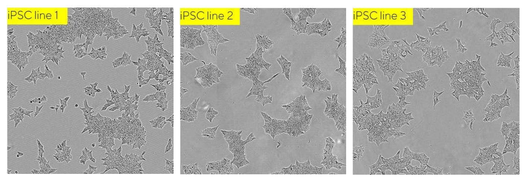
Test pluripotency

Using a combination approach, cell surface markers and morphological indicators of pluripotency can be analyzed using the iQue® Advanced Flow Cytometer and the Incucyte® Live-Cell Analysis System to identify the optimal line for expansion and downstream experiments.

Cell type	Expected pluripotency?	Reprogramming method	Somatic cell source
THP-1	N	N/A	Monocytic leukemia
NCCIT	Y	N/A	Mediastinum
iPSC line 1	Y	Episomal	Cord blood
iPSC line 2	Y	Sendai virus	Foreskin fibroblasts
iPSC line 3	Y	Sendai virus	Hepatic fibroblasts







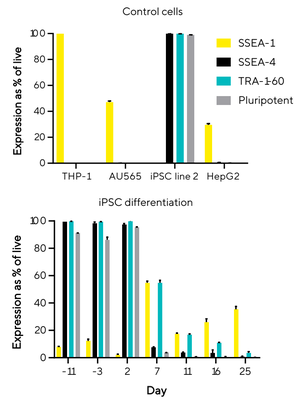
- Cells were stained for the following surface markers, SSEA-1 (non-pluripotency), SSEA-4 and TRA-1-60 (pluripotency) and also gated for a pluripotent population (SSEA-1 -, SSEA-4 +, TRA-1-60 +).
- 3 lines were tested for use in further experiments, iPSC line 2 was chosen based on viability (Membrane Integrity Dye), expression profile and morphological characteristics.

Monitor differentiation

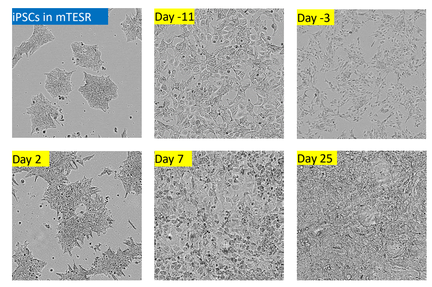
Follow differentiation (e.g. to iPSC-derived hepatocytes) by monitoring pluripotency and target cell type marker expression using the iQue® and morphology over time using the Incucyte®.

- The iQue® Advanced Flow Cytometer provides the tools for monitoring surface marker expression during differentiation workflows.
- iPSCs lose pluripotent expression markers during differentiation into hepatocyte-like cells.
- Marked morphological changes can be monitored using the Incucyte® Live-Cell Analysis System as iPSCs differentiate into 'hepatocytes'.

Differentiation performed using the Cellartis iPS Cell to Hepatocyte Differentation System

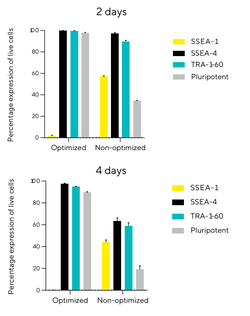


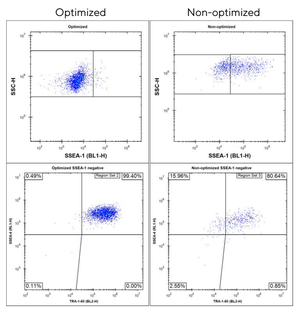
Cell type	Expected pluripotency?	Cell line description
THP-1	N	Monocytic leukaemia
AU565	N	Breast cancer
iPSC line 2	Y	iPSC
HepG2	Y	Liver cancer

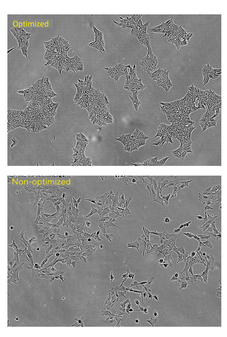


Monitor 2D growth

Culture iPSC cells in 2D and test the effects on pluripotency and morphology of different media formulations – image on the Incucyte® and stain for surface markers on the iQue®.





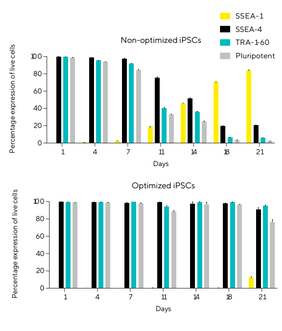


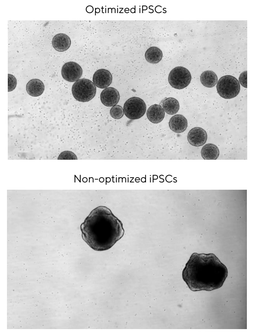
- iPSCs grown in non-optimized conditions display reduced expression of key pluripotent markers with increased expression of non-pluripotent markers.
- Morphological differences are readily apparent through imaging on the Incucyte®, analysis of which highlights decreased nuclear/cytoplasm ratio in non-optimized conditions.

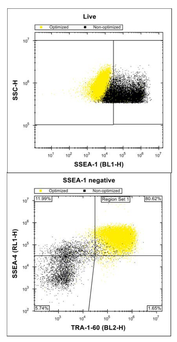
Optimized = mTESR Plus, Non-optimized = RPMI
iPSCs grown on Vitronectin XF coated culture plates

Monitor 3D growth

Culture iPSC cells in 3D long term in shake flasks, analyzing the effects of different culture conditions over time – image on the Incucyte® and stain for surface markers on the iQue®.







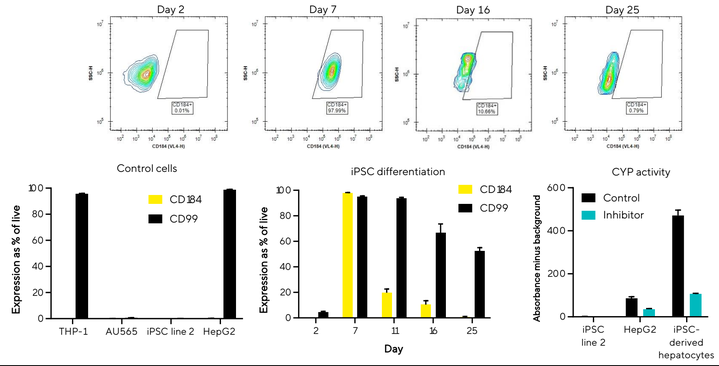
- iPSCs grown in 3D over 21 days in non-optimized conditions display reduced pluripotent marker expression with time.
- Incucyte® images of 3D iPSC spheroids highlight the changes in morphology associated with loss of pluripotency, spheroids become larger with increased eccentricity.
- iQue Forecyt® software provides solutions for data presentation that is clear and concise, showing the clear differences in expression profile of optimized and non-optimized culture conditions in iPSCs using multicolor dot plots.

Optimized = regular passaging, Non-optimized = no passaging
3D spheroids grown in Stemscale PSC Suspension Medium

Assess derived cell function

Use functional assays to determine whether cell has been differentiated to the desired state (e.g. luminescence based Cytochrome P450 (CYP) inhibition assay to assess hepatocyte function).

- During the definitive endoderm (DE) stage of hepatic differentiation, iPSC derived cells gain (day 7) and then lose (day 16+) expression of CD184, a key marker of DE.
- iPSCs differentiated into mature hepatocyte-like cells display increased expression of hepatic marker CD99.
- Functional testing of iPSC derived mature 'hepatocytes' illustrates high levels of enzymatic activity associated with hepatic tissue.



RUO growth factors and cytokines

Maintain iPSC pluripotency without the requirement for daily feeding.

- iPSC cells were cultured without media renewal for 5 days using NutriStem® hPSC XF Growth Factor (GF)-Free supplemented with Sartorius® Recombinant TGF-β1 PLUS protein (2 ng/mL) and FGF2-G3 protein (50-200 ng/mL), a thermostable variant of FGF-2.
- Supplementation with FGF2-G3 maintains pluripotent marker expression in iPSCs without daily feeding.
- iPSCs supplemented with FGF2-G3 display normal tightly packed colony morphology with minimal cell death when compared to controls without supplementation.