Qkine

Application note

Weekend-free human induced pluripotent stem cell culture using thermostable FGF-2 (bFGF) and animal origin-free TGF-β1 for improved colony homogeneity

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Feeder-free human induced pluripotent stem cells (iPSCs) culture is a highly repetitive and routine process that can be costly due to the amount of investment required for media and the required frequency of media changing. The additional cost for media is based around the need to have two core intercellular communication growth factors, Fibroblast growth factor 2 (FGF-2) and transforming growth factor β1 (TGF-β1) present at precise concentrations to maintain their pluripotency. However, to achieve this in an *in vitro* setting can be challenging due primarily to the rapid degradation of FGF-2, which has an effective half-life of <10h, resulting in the requirement for daily media changes.

In this application note, we describe the method for maintaining, growing, and testing the pluripotency of iPSCs using E8-like media containing Qkine thermostable FGF2-G3 (Qk053) and TGF-β1 PLUS (Qk010) growth factors. This process showcases the maintenance, expansion, and pluripotency evaluation of iPSCs without the need for daily feeding, enabling weekend-free maintenance and expansion while preserving the cells' pluripotency and proliferative potential.

Introduction

Human induced pluripotent stem cells (iPSC) are an *in vitro* model that are functionally immortal and have the capacity to proliferate while maintaining the ability to, hypothetically, differentiate to any somatic cell type. Interest in iPSC generation has grown significantly because they can be used across various research areas and fields without facing the same ethical concerns associated with the use of embryonic stem cells.

This has created a need to culture large quantities of individual iPSC lines or a diverse array of different iPSC lines. Consequently, this has increased the cost and time-consuming demand for daily media changes. This demand has driven the effort to identify the core signalling components required in the cell culture medium to efficiently culture iPSC. FGF-2, which binds with FGFR1/FGFR4, activating the PI3K/AKT/mTOR pathway and TGF-β1, which binds with TGFBR1/2 to activate the TGF-β, have been highlighted as two of these core signalling components.

Maintaining the pluripotency during routine maintenance is a challenge with cell cultures known for their sensitivity such as iPSC. Using high purity and thermostable growth factors can help reduce this risk as they reduce the impact on genotypic stability as they lack impurities such as endotoxins and provide a more consistent exposure to necessary growth factors.

E8-like media is a chemically defined media, meaning that it contains less undefined components that are found in other complex media. This results in reduced variability and improves the reproducibility of the culturing between individual iPSC line or a variety of different iPSC lines. This formulation has been optimized to support robust cell proliferation while ensuring the pluripotency of iPSC remains and resulting in improved cell yields and more consistent scaling up of cultures. Pluripotency of iPSCs can be evaluated by investigating intracellular surface markers such as NANOG, SOX2 and OCT-4.

Materials and methods

The iPSC routine culturing workflow schematic (Figure 1) outlines the steps for the maintenance of iPSC on a day-by-day basis and demonstrates the difference against using the standard protocol. The pluripotency evaluation schedule highlights the key points in the timeline for testing the retention of iPSC pluripotency

(Figure 2).

Cell culture and maintenance

iPSC were passaged twice per week using 0.5mM EDTA for detachment and seeded in vitronectin (5µg/ml) coated 6-well plates using a 1:6 split ratio and cultured in an E8-like media (Table 1). The day after passaging, spent media was removed to be replaced with 5ml

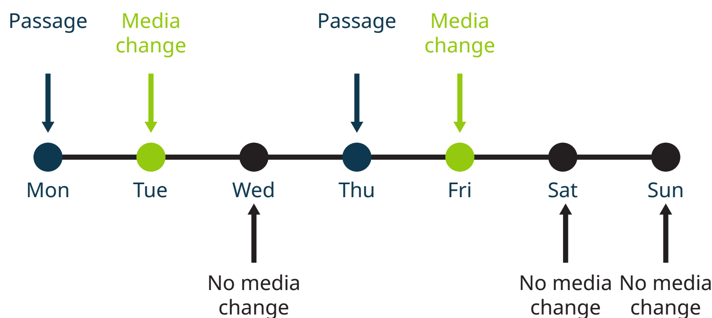

Figure 1: Weekly schedule for routine maintenance of iPSC

Component	Final Concentration
DMEM/F12 medium (Thermo Fisher Scientific,31330038)	1x
Sodium bicarbonate (Merck, S5761)	1086 mg/L
Sodium chloride (Merck, S5886)	2000 mg/L
L-ascorbic acid (Merck, A8960)	128 mg/L
Insulin-Transferrin-Selenium (ITS-G) (Thermo Fisher Scientific, 41400045)	Human EGF protein
FGF2-G3 (Qkine, Qk053)	40 ng/ml
TGF-β1 PLUS (Qkine, Qk010)	2 ng/ml

Table 1: E8-like media construction components. Components added aseptically and filter sterilised before use.

Immunocytochemistry (ICC)

iPSC were passaged using AccutaseTM for detachment and seeded at 500 cells/well in a Vitronectin (5µg/ml) coated 96-well plate in E8-like media containing ROCK inhibitor (Y-27632, 10µM). The day after seeding, the ROCK inhibitor was removed and 200µl of E8-like media was added to each well. After 3 days, cells were fixed with 4% Paraformaldehyde, blocked and permeabilized with 10% donkey serum diluted in 0.1% Triton X-100. Specific antibodies for markers of pluripotency (OCT-4, NANOG and SOX2) were then used to immunostain the cells overnight at 4oC. iPSCs were then washed and incubated with secondary antibody Donkey anti-Mouse AlexaFluorTM 647 or Donkey anti-Goat AlexaFluorTM 488 and Hoechst 33258, followed by imaging in phosphate buffered saline (PBS). Brightfield and fluorescence images were acquired using the EVOS M5000 system.

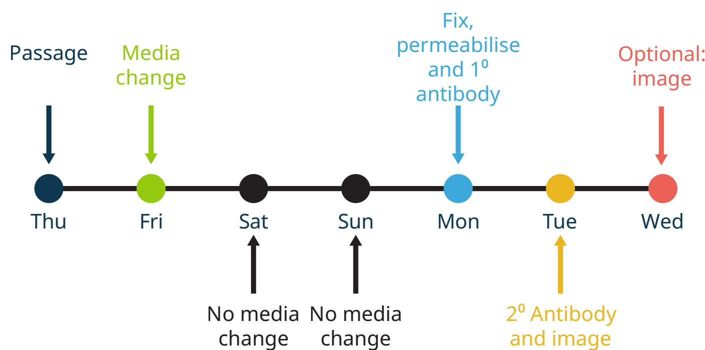

Figure 2: Schedule for pluripotency evaluation testing

Results

FGF2-G3 and TGF-β1 PLUS maintain pluripotency, morphological appearance and marker expression in iPSC

iPSC cultured for a month in E8-like media containing Qkine FGF2-G3 and TGF-β1 PLUS growth factors, consistently demonstrated high confluency without spontaneous differentiation. Brightfield imaging demonstrated a highly retained good cell morphology, well-rounded colonies with defined edges and compacted cells, over this time course (Figure 3). These iPSC retained their pluripotent potential by expressing high levels of the pluripotency markers NANOG, SOX2 and OCT-4, as shown in Figure 4.

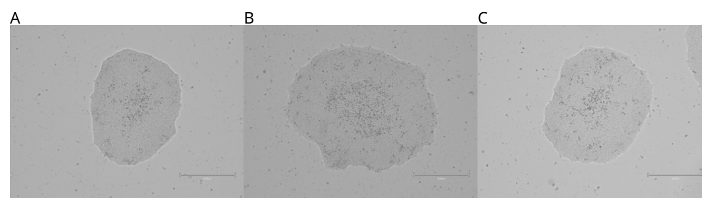

Figure 3: Imaging of the highly retained morphological appearance of colonies grown in E8-like media. (A) after 2 passages and 7 days in culture (B) After 4 passages and 14 days in culture (C) After 8 passages and 28 days in culture (scale bar = 300µm).

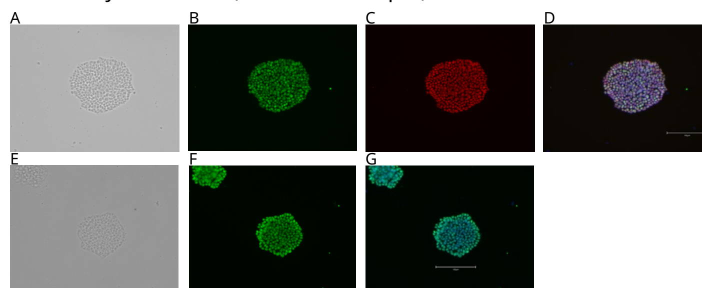

Figure 4: ICC of pluripotency markers in iPSC grown in E8-like media. Brightfield image (A), NANOG expression (green, B), OCT-4 expression (red, C), combined NANOG, OCT-4 and Hoechst 33258 (D). Brightfield image of stained colony (E), SOX2 expression (green, F) combined SOX2 and Hoechst 33258 (G, blue) scale bar = 150µm.

Conclusion

The importance of iPSC in clinical and research fields is increasing due to their potential to differentiate into all somatic cell types in the human body. Successful differentiation depends on maintaining the pluripotency of iPSC during culture, making the use of the correct media crucial.

The data presented in this application note demonstrate that using Qkine FGF2-G3 and TGF-β1 PLUS thermostable growth factors in an E8-like media preserve iPSC pluripotency and support proliferation while preventing the need for daily media changes.

Further information

These iPSC have then been differentiated into various germ layer and cell types, further indicating the fact that their pluripotency is maintained using our weekend-free media change conditions in combination with our growth factors.

When compared to iPSC cultures grown in an E8-type media, a similar proliferation rate is observed but there is a higher level of spontaneous differentiation, lowering the quality of the iPSC colonies present for future work. These cells also required daily media exchange, resulting in higher cost and time investment in maintaining the cultures (data available upon request).

Qkine growth factors are manufactured to the highest of quality standards and are free from animal-derived contaminants, delivering low endotoxicity and high purity. At Qkine, we are committed to raising the standards of growth factors, cytokines and related proteins to better support long-term and complex neural stem cell culture. We are a science-led team, please reach out with any questions or requests to support@qkine.com

For more information

Please contact our team: **customerservice@qkine.com** if you would like to discuss commercial or academic collaborations, supply agreements or any aspects of growth factor optimization and other products.

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