A Novel Essential 8™ Media System for Robust Weekend-Free Culture of Pluripotent Stem Cells

Matthew R. Dallas[1], Jeffrey A. Fein[1], Majidat A. Muhammad[1], Chetana M. Revankar[2], Jonathan F. Zmuda[1]

[1] Thermo Fisher Scientific, 7335 Executive Way, Frederick, MD; [2] Thermo Fisher Scientific, 5781 Van Allen Way, Carlsbad, CA

ABSTRACT

Pluripotent stem cells (PSCs) are powerful tools for developmental biology, regenerative medicine, and the study of debilitating human diseases. While the development of feeder-free culture systems has significantly simplified routine PSC culture, additional hurdles continue to challenge today's stem cell scientists. Prominent among these is the daily media exchange required to maintain healthy PSC cultures. This daily feeding not only necessitates seven day workweeks for scientists but also increases the potential for contamination and introduces variability resulting from multiple users processing a single culture. The requirement for daily feeding is driven largely by the heat sensitivity of key factors crucial to the maintenance of pluripotency in culture, with activity loss beginning immediately upon warming of growth medium and deteriorating upwards of 80-90% after 24 h at 37°C. In this work we have developed a new Essential 8 (E8)-based cell culture media that allows for long-term, feeder-free, healthy PSC culture without the need for weekend feeding. In this new culture medium virtually no loss of bioactivity is observed in key heat-labile components, including FGF2, at 37°C for periods of 72 h or greater. The medium has been used with equal success in embryonic and induced pluripotent stem cell culture and does not require low cell densities or other significant changes to current culture workflows. Furthermore, our data indicate that PSCs cultured in this formulation using a weekend-free culture schedule (1) maintain expression of canonical pluripotency markers, including Nanog and TRA-1-60, (2) retain the capacity for trilineage differentiation, and (3) exhibit a normal karyotype over long-term passaging. In addition to simplifying and securing day-to-day PSC culture, enhanced stability of key factors will have added benefits in large-scale cultures for regenerative medicine, where large volume media exchanges are impractical and consistent performance is critical.

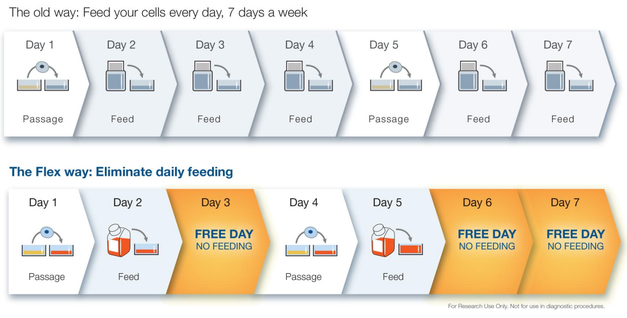


FIGURE 1. The extended bioactivity of key PSC media components in Essential 8 Flex allows for routine weekend-free culture of healthy stem cell cultures. Unlike with other alternative feed protocols, which often require very low split ratios, Essential 8 Flex weekend-free culture is compatible with a standard, twice-weekly split schedule.

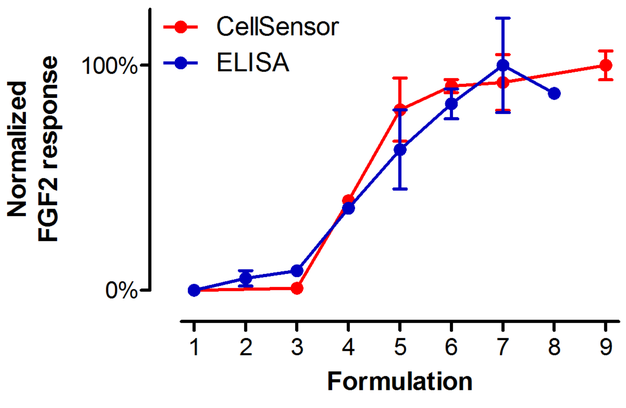
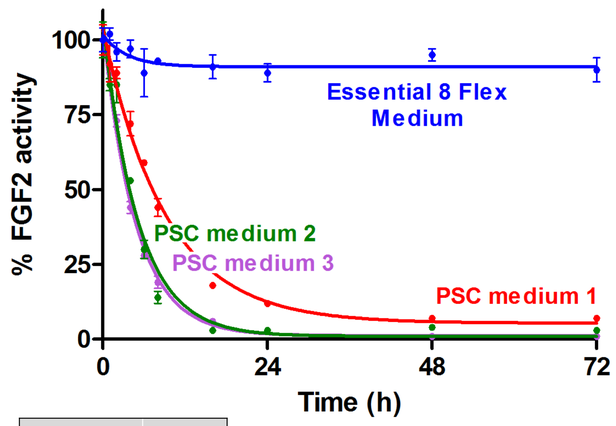
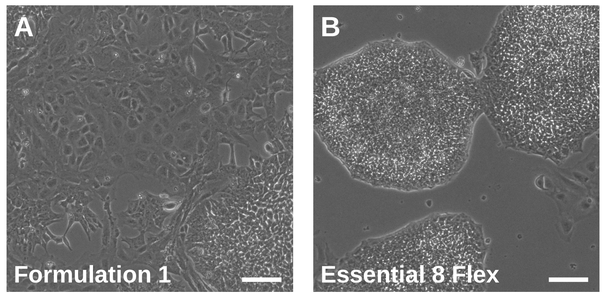


FIGURE 2. FGF2 bioactivity after 24 h at 37°C was measured in a series of Essential 8-based prototype formulations using a cell-based bioassay (CellSensor c-fos-bla, K1111; red) and by ELISA (Novex, KHG0021; blue). The optimized formulation number 7, which became the final Essential 8 Flex formulation, maximized FGF2 activity as measured in each assay.



Media	$t_{1/2}$
Essential 8 Flex	146 h
PSC medium 1	8 h
PSC medium 2	5 h
PSC medium 3	5 h

FIGURE 3. FGF2 bioactivity in various PSC culture media was measured over periods between 1 and 72 h using the aforementioned ELISA. Curves were calculated using a standard one-phase decay model for all media. FGF2 activity levels at 24 h were then used to calculate an expected half-life of FGF2 in each media tested (left).



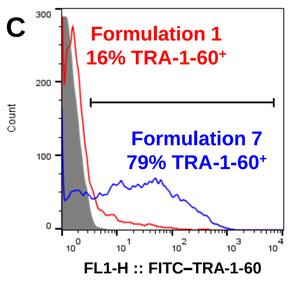


FIGURE 4. As an additional test of FGF2 stability in the optimized formulation, prototype formulation number 1 (*A*) and Essential 8 Flex (*B*) were incubated at 37°C for a period of 48 h before routine PSC culture. Scale bars, 100 µm. *C,* Flow cytometry was then used to measure TRA-1-60 expression as an indicator of pluripotency.

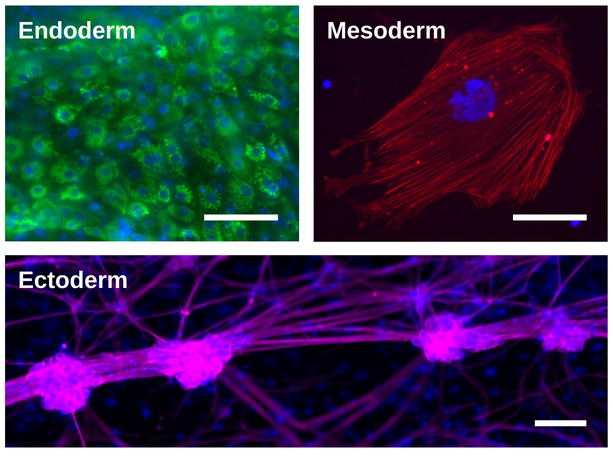


FIGURE 5. Embryoid bodies formed from passage 15 weekend-free H9 ESCs 15 were allowed to spontaneously differentiate on LDEV-free Geltrex™ matrix (Gibco, A1413301). The Molecular Probes™ 3-Germ Layer Immunocytochemistry Kit (A25538) was used to identify cells of each of the three germ lineages: endoderm (α-fetoprotein; upper left panel), mesoderm (smooth muscle actin; upper right panel), and ectoderm (βIII-tubulin; lower panel). Scale bars, 100 µm.

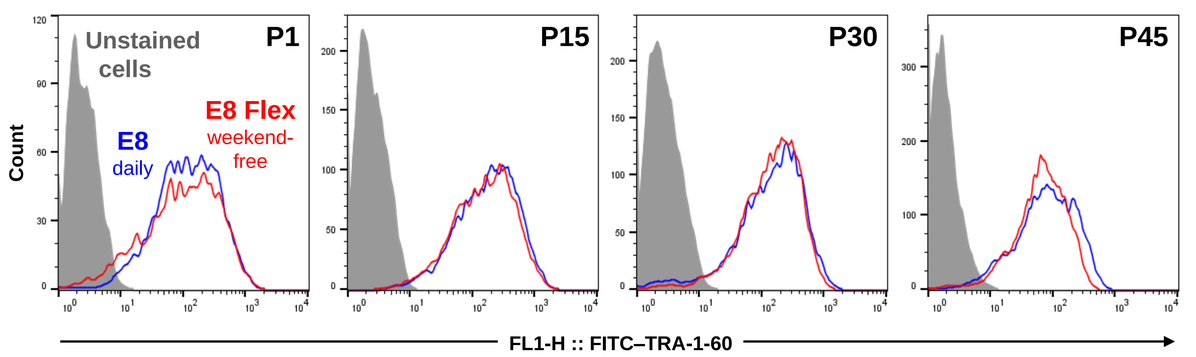


FIGURE 6. Flow cytometry was used to monitor TRA-1-60 expression in the Gibco episomal iPSC line during long-term culture. Cultures were split twice weekly and either fed daily with Essential 8 (E8; blue histograms) or fed with Essential 8 Flex using the modified, weekend-free feed protocol laid out in Fig. 1 (red histograms).

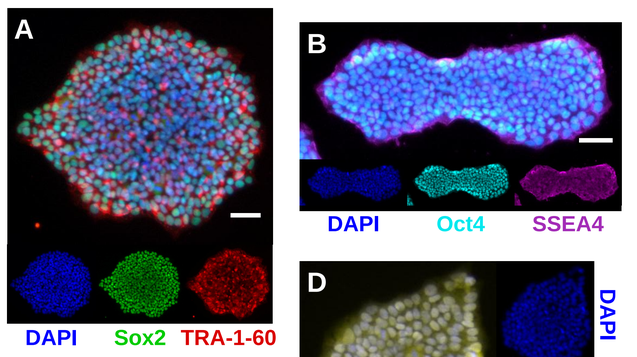


FIGURE 7. Pluripotency marker expression during long-term, weekend-free passaging was assessed via immunocytochemistry using the EVOS™ FL Auto Imaging System (AMAFD1000). *A-C,* Sox2, TRA-1-60, Oct4, and SSEA4 expression were shown using the Molecular Probes Pluripotent Stem Cell 4-Marker Immunocytochemistry Kit (A24881). *D,* Nanog expression was demonstrated using the Pierce™ anti-Nanog primary (PA1-097) and Alexa Fluor™ 488-conjugated secondary (Molecular Probes, A11008) antibodies. Artificial color has been added to distinguish individual channels; scale bars, 50 µm.

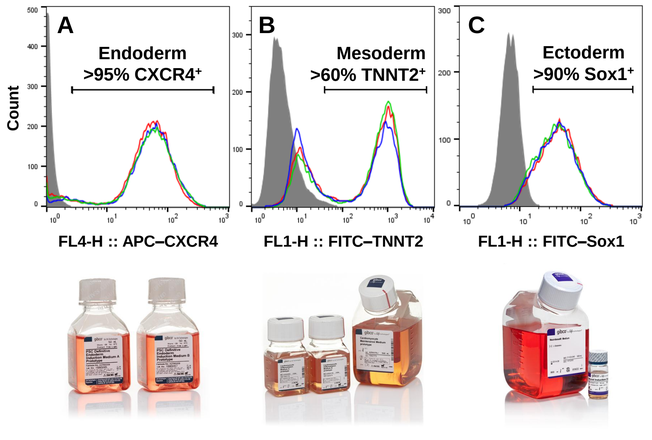


FIGURE 8. Directed differentiation efficiency was assessed in Gibco episomal iPSC (*A, C*) and H9 ESC (*B*) cultures passaged ≥5 times in Essential 8 Flex with weekend-free feeding. The Gibco PSC Definitive Endoderm Induction Kit (A27654SA), Neural Induction Medium (A1647801), or Cardiomyocyte Differentiation Kit (A25042SA) was used to generate specific cell types. Differentiation efficiency was then evaluated via flow cytometry using CXCR4, TNNT2, and Sox1 expression as indicators of definitive endoderm (*A*), cardiomyocyte (*B*), and neural stem cell (*C*) differentiation, respectively. Gray histograms indicate unstained cells used as negative controls.

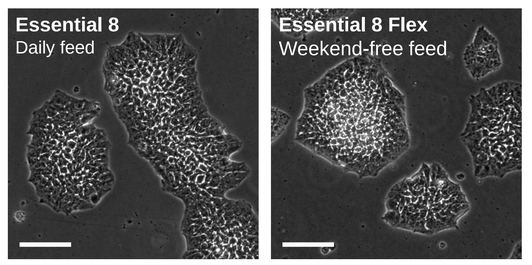


FIGURE 9. Standard PSC morphology is observed in long-term Essential 8 Flex cultures without weekend feeding. Scale bars, 100 µm.

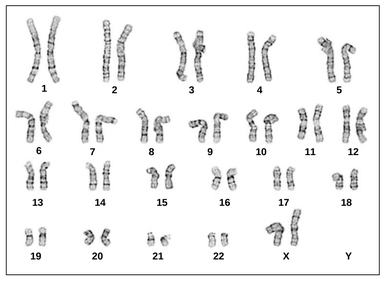


FIGURE 10. H9 ESC and Gibco episomal iPSC karyotypes were monitored over the course of long-term culture in Essential 8 Flex Medium. Stable, normal karyotypes have been observed to at least 30 passages.

CONCLUSIONS

The optimized Essential 8 Flex formulation effectively extends the bioactivity of key pluripotency-associated growth factors, including FGF2, for periods 72 h or greater. This prolonged activity allows for Essential 8 Flex Medium to support long-term, weekend-free pluripotent stem cell culture in which PSC morphology, pluripotency marker expression, tri-lineage differentiation potential, and normal karyotypes are all preserved.

ACKNOWLEDGEMENTS

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