DEVELOPMENT OF A NOVEL XENO-FREE MEDIUM FOR FEEDER-FREE CULTURE OF HUMAN STEM CELLS

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ABSTRACT

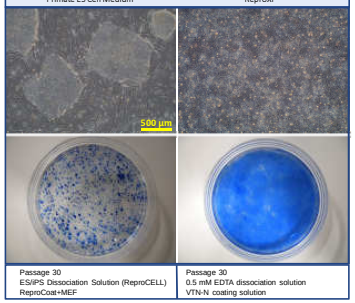
Culture of human ES cells and iPS cells has attracted a lot of interest due to the applications of stem cells in both drug screening as well as regenerative medicine.

Most researchers co-cultivate human ES or iPS cells on mouse derived MEF feeder cells. However, presence of the feeder cells could affect the application of the resulting stem cell to other areas of research. Moreover, the current conventional culture medium may contain animal-derived serum; this may increase the risk trans-species infection from implantation of stem cells or stem-cell derived materials. Such issues could cause a setback in the clinical application of stem cell research. In order to solve such issues, we have developed a new culture medium, ReproXF™, which does not contain any animal-derived components. Culture with ReproXF™ allows researchers to cultivate human iPS and ES cells under feeder-free conditions without compromising the quality.

Here, we demonstrate that human iPS cells cultivated using ReproXF™ showed alkaline phosphatase activity. Furthermore, these iPS cells showed strong expression of the pluripotency markers OCT3/4, NANOG, SSEA-1, TRA1-60 and TRA1-80 by both immunostaining and flow cytometry. Also, from the result of immunostaining, we have confirmed that the karyotype of these iPS cells is normal. In addition, we also confirmed that human iPS cells cultivated by ReproXF™ possess the ability to differentiate into neurons and cardiomyocytes. Taken together, these data show that ReproXF™ medium not only allows human iPS cells to remain in the undifferentiated, pluripotent state, but also to retain the ability to differentiate under standard conditions.

In conclusion, we believe that ReproXF™ medium will allow researchers to produce large amounts of high quality of human ES or iPS cells for use in regenerative medicine and basic research.

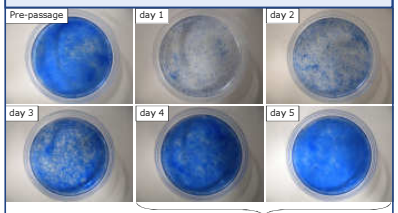
Culture of Human 201B7 iPS Cells in ReproXF Yields High-Density Cultures



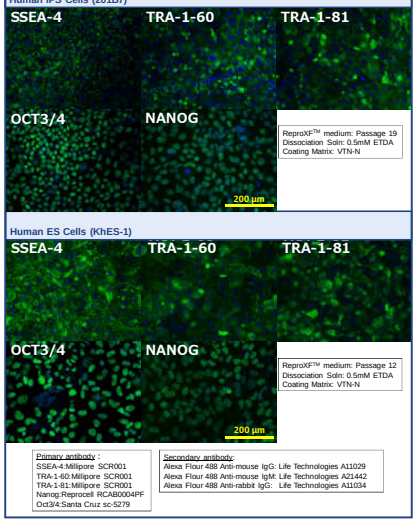
ReproXF™ Supports the Use of a Variety of Passaging Conditions

Dissociation Solution	Laminin-5 (ReproCell)	VTN-N (LTI)	Matrigel (Corning)	Synthemax-II (Corning)
0.5 mM EDTA	OK	OK	xx	+/-
Accutase	OK	OK	OK	OK

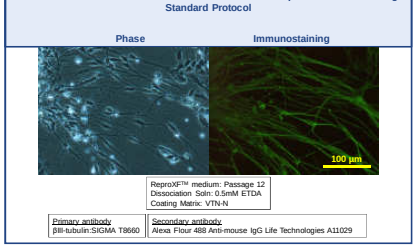
ReproXF™ Supports a Five-Day Passage Cycle



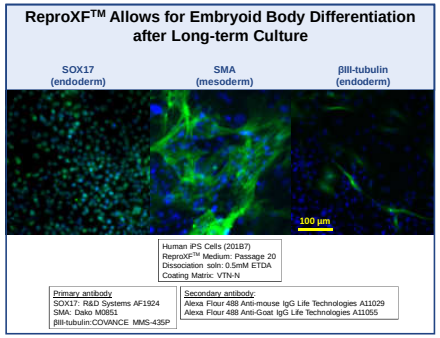
ReproXF™ Medium Supports Long-term Culture of Undifferentiated Human iPS and ES Cells



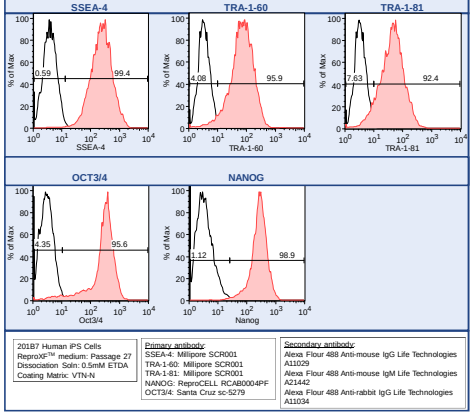
ReproXF™ Supports Differentiation into Neurons



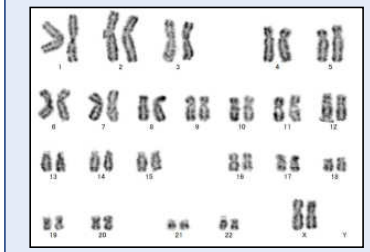
ReproXF™ Allows for Embryoid Body Differentiation after Long-term Culture



ReproXF™ Medium Supports Long-Term Culture of Undifferentiated Human iPS Cells



Long-term, High-density Culture of Human iPS Cells in ReproXF™ Medium Results in Normal Karyotype



Conclusions

- We have developed ReproXF™, a new xeno-free medium for high-density, feeder-free culture of human iPS and ES cells.
- After long-term culture in ReproXF™, human iPS and ES cells remain in an undifferentiated state, expressing common markers.
- After long-term culture in ReproXF™, human iPS cells retain the ability to differentiate upon receiving the appropriate signals.
- ReproXF™ medium allow for long-term, economical production of stem cells.