Human pluripotent stem (ES/iPS) cells Xy tech BOF-

R&D; Bourbon Biomedical Advanced Research Laboratories, Inc.



72hr change-free medium culture of human ES/iPS cells in Xyltech BOF-01

- •BOF-01 is a novel basal culture medium which can suppress proliferation of human ES/iPS cells on feeder cell lavers.
- •BOF-01 can be used by completely replacing the basal culture medium (e.g. DMEM/F12) of the human ES/iPS cells.
- Human ES/iPS cells can be maintained with BOF-01 for about 3days (up to 72 hours) without changing medium under normal culture conditions (37°C, 5% CO2).
- •After suppression of cell proliferation with BOF-01, cell growth can be resumed by changing normal growth medium.
- •This product does not contain glucose.

- It cannot be used under feeder-free culture conditions
- BOF-01 does not contained growth factors nor supplements (such as KSR, bFGF etc.).It is required to add necessary factors appropriately to prepare complete culture medium.
- Not all human ES/iPS cell lines have been tested with this product.
- None of non-stem cell lines are profiled either.
- This product does not inhibit cell proliferation completely.
- Cellular condition under BOF-01 culture depends on the state and culture condition of the human ES/iPS cells.
- This product is for research use only, and not permitted for human or animal diagnostic or therapeutic uses

Overview of Weekend-free Protocol

THU

• Cell proliferation control by BOF-01 made it possible to maintain/preserve cells during the weekend in a normal culture environment without changing the medium. Cell Passaging

TUE

Cell maintenance with proliferation control (No medium change) FRI

SUN



Week 1

Maintain cell culture, experiments, repeat cell passaging schedule with BOF-01 medium on Friday

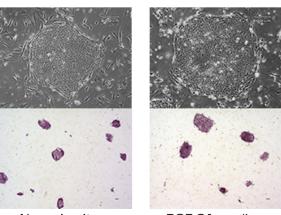
Week 2 TUE MON

MON

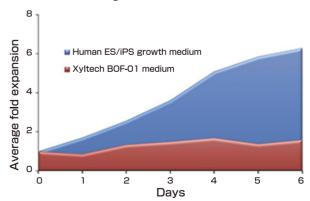
WED THU FRI The timing of passage needs to be adjusted to the cell's condition and growth rate.

Morphology of Undifferentiated Cell Colony and Effect of Proliferation Control

 Phase images of cell colonies and alkaline phosphatase stain of human iPS cells before and after proliferation control culture by BOF-01 (201B7 strain).



• Comparison of cell proliferation rates of hiPS cells cultured in BOF-01 medium and normal human ES/iPS growth medium.



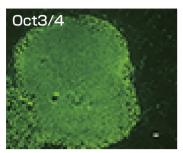
- *The composition of normal growth medium (human ES/iPS medium) and BOF-01 medium are based on reference.
 - Curr Protoc Stem Cell Biol.2009 Jun; Chapter 4: Unit 4A.2.

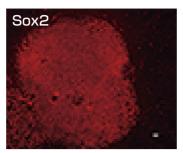
Normal culture BOF-01 medium

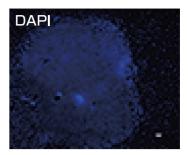
Xyltech BOF-01 suppressed cell proliferation while maintaining human iPS cells in undifferentiated state.

Undifferentiation markers expression in human iPS cells by BOF-01

•The high expression of pluripotent stem cell markers was confirmed by immunofluorescence staining of human iPS cells (201B7) maintained in BOF-01 medium for 3 days.



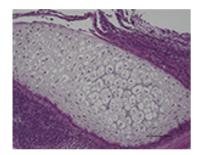




Xyltech BOF-01 maintained human iPS cells in undifferentiated state.

Differentiation of Three Germ Layers (Teratoma Assay)

•The ability of differentiation was confirmed by transplanting into immunodeficient mouse by human iPS cells (201B7) maintained in BOF-01 medium for 3 days to form teratoma.



Mesoderm (Chondrocyte)



Endoderm (Alimentary canal epitherial cell)

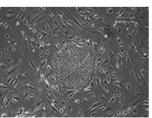


Ectoderm (Pigment cell)

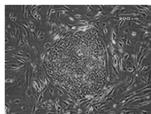
Xyltech BOF-01 maintained human iPS cells in pluripotent state.

Colony morphology changes during proliferation control culture

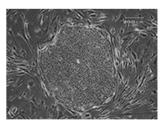
HumanES/iPS cells may change the morphology of colonies while cultured in BOF-01 medium. The original morphology can be resumed after changing back to normal human ES/iPS medium (Phase images show the example of 201B7 cells).



Normal culture (Day 1)



BOF-01 medium (Day 4)



Back to normal culture (Day 5)

Cat. No.	Product name	Expiration	Storage	Volume
10101	Xyltech BOF-01	12 months	2~8℃	100mL

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