Proliferation Control Medium for Basic Culture of Human Pluripotent Stem Cells

Xyltech BOF-01

User's Guide

Ver. 4.4

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Protocol: Human induced pluripotent stem (iPS) cell culture proliferation control using Xyltech BOF-01

1. Product features

Xyltech BOF-01 is a maintenance medium for human pluripotent stem cells (hPSCs) grown on a feeder layer of cells.

Xyltech BOF-01 (BOF-01) suppresses the proliferation of hPSCs, maintaining them in an undifferentiated state. BOF-01 can be used by completely replacing the basal culture medium (e.g. DMEM/F12) of the hPSCs. The cells can be maintained with this medium for about 3 days (up to 72 hours, without changing the medium) under normal culture conditions (37°C, 5% CO_2).

After suppression of cell proliferation with BOF-01 medium, cell growth can be resumed by changing back to normal growth medium.

This product does not contain glucose.

2. Precautions

This product, BOF-01, is a basal medium for hPSCs grown on feeder cells. It cannot be used under feeder free culture conditions.
This product is for research purposes only, and is not intended for use in the treatment or diagnosis of humans or animals.

3. Preparation method

Replace the basal cell culture medium with BOF-01. An example of the composition of normal human ES/iPS medium and BOF-01 medium is described in section 4-1. References are listed in section 7.

4. Method for cell culture: Example of human ES/iPS medium and BOF-01 medium

4-1. Cells and Reagents

- Human iPS cells (grown on feeder cells, 60mm culture dish)
- Feeder Cells: Mitomycin C (MMC) treated mouse embryonic fibroblast (MEF) (60mm culture dish)
- Xyltech BOF-01
- DMEM/F12
- KnockOut Serum Replacement (KSR)
- Non-Essential Amino Acid Solution (NEAA)
- L-Glutamine
- 2-Mercaptoethanol
- Penicillin/Streptomycin
- DPBS without calcium and magnesium ($DPBS^{--}$)
- Dissociation solution for human ES/iPS Cells (Collagenase Type IV, Trypsin, KSR: CTK solution)

Normal Growth Medium (Human ES/iPS Medium)	BOF-01 Medium
DMEM/F12	**BOF-01**
20% KSR	20% KSR
0.1 mM NEAA	0.1 mM NEAA
2 mM L-Glutamine	2 mM L-Glutamine
0.1 mM 2-Mercaptoethanol	0.1 mM 2-Mercaptoethanol
50 units/ml Penicillin	50 units/ml Penicillin
50 mg/ml Streptomycin	50 mg/ml Streptomycin
4 ng/ml bFGF	4 ng/ml bFGF

4-2. Proliferation control of human iPS cells with BOF-01

1. Prepare Xlytech BOF-01 medium, (see composition in example above) and prewarm in a 37°C water bath.
2. After 1 to 3 days passage, select human iPS cells which are relatively small in colony size (up to 50 cells per colony), remove the culture medium and replace with 5 ml of BOF-01 medium.*
3. Maintain the cells in a 5% CO_2 incubator for up to 3 days. There is no need to change the BOF-01 medium over this time period.
4. After up to 3 days of proliferation-controlled culture with BOF-01 medium, replace the medium with human ES/iPS normal growth medium and continue to culture the cells overnight.
5. The next day, check the density and morphology of cell colonies with a phase contrast microscope before proceeding with cell culture and experiments.

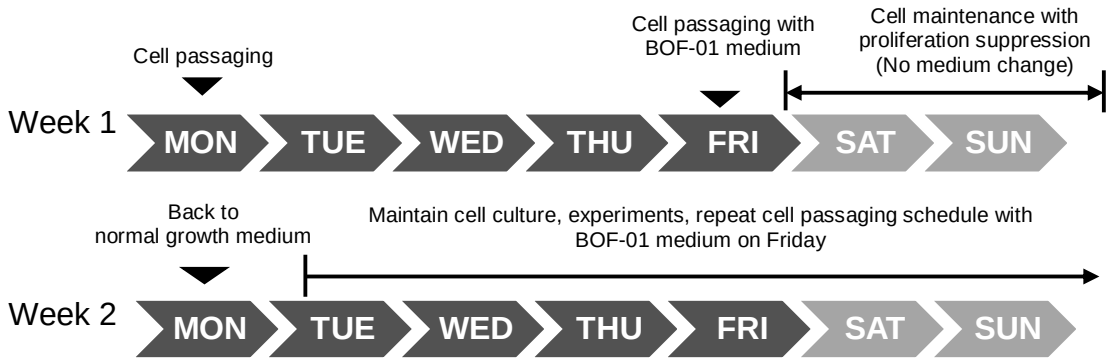
* We recommend using relatively small sized colonies to maintain the culture for up to 3 days. If the cell density is high (over 60-70% confluency), it is recommended to replace BOF-01 medium with fresh BOF-01 medium earlier than 3 days to avoid the potential increase of floating cells.

4-3. Passaging and proliferation control of human iPS cells (weekend-free culture method)

1. Warm BOF-01 medium, $DPBS^{--}$ and dissociation solution, in a 37°C water bath.
2. Remove culture medium from confluent human iPS cells.
3. Add 2 ml of $DPBS^{--}$ to wash the cells, then remove the $DPBS^{--}$.
4. Add 0.5 ml of dissociation solution and incubate at 37°C for 2-5 minutes.
5. Remove the dissociation solution, add 2 ml of $DPBS^{--}$ to wash the cells then remove the $DPBS^{--}$.
6. Add 2 to 4 ml of BOF-01 medium to the cells, pipette slowly, several times, then dislodge the iPS cell colonies from the feeder layer, and make clumps of 20-30 cells.
7. Remove the culture supernatant from a 60mm culture dish containing a new feeder layer of MMC treated MEF cells and add the clumps of iPS made in step 6 by diluting them in BOF-01 medium (for example make a 5 fold dilution by adding 1 ml of cell clump suspension to 4 ml of BOF-01 medium) and seeding them over the feeder cells. Gently shake the dish to seed the cell clumps uniformly.
8. Maintain the cells without changing the BOF-1 medium for (up to) 3 days in a 37°C, 5% CO_2 incubator.

9. After 3 days, remove BOF-01 medium from the human iPS cells, then add human ES/iPS medium.
10. On the following day, observe the cells with a phase contrast microscope, mark colonies that tend to differentiate, and remove by suction. After changing back to human ES/iPS medium, change medium every day. Cells are typically subcultured between a 1:3 (3 fold dilution) and 1:6 (6 fold dilution) ratio, and every 4 to 5 days under normal growth conditions.

Below is an example of the schedule for the weekend-free culture method.

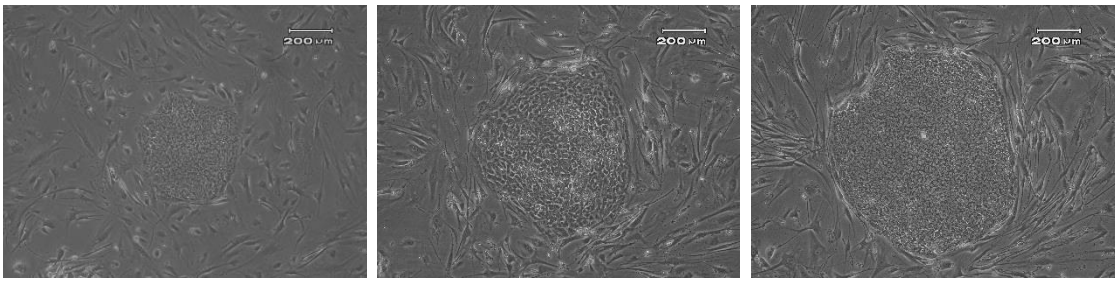


*Cultured human iPS cells with normal growth medium (human ES/iPS medium) are passaged on Friday afternoon using BOF-01 medium. Cells can be in continuous culture without replacing the BOF-01 medium during the weekend, then changing back to the normal growth medium on Monday morning.

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

5. Additional information

Human 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 growth medium (Phase images show example of an 201B7 cells).



Before changing to BOF-01 medium

Cells maintained with BOF-01 medium

24 hours after changing back to human ES/iPS medium

6. References

1) Curr Protoc Stem Cell Biol. 2009 Jun; Chapter 4: Unit 4A.2.
2) Protocol of Kyoto University iPS Laboratory (CiRA); Revision Ver.2, March 5, 2009
   "Generation of Human Induced Pluripotent Stem Cells"
   https://www.cira.kyoto-u.ac.jp/e/research/img/protocol/hipsprotocolv2_090304.pdf

7. Product inquiries

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* The protocols shown here are based on our experimental experience. Adjustments to the seeding density, and passage timing may be required according to the cell line of your usage. This product (Xyltech BOF–01) is intended for research purposes only, and is not permitted for use in the treatment or diagnosis of humans or animals.