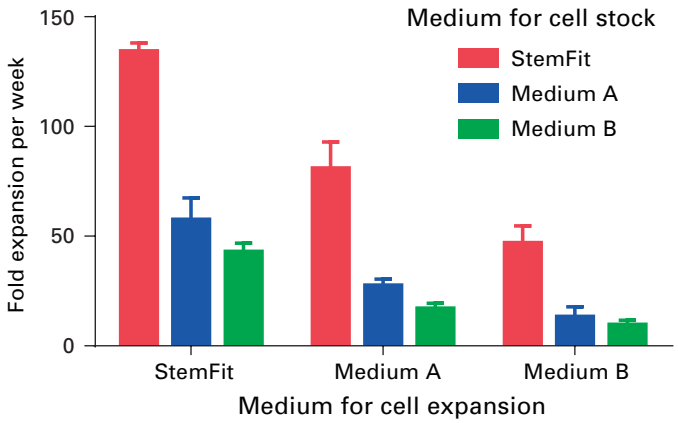
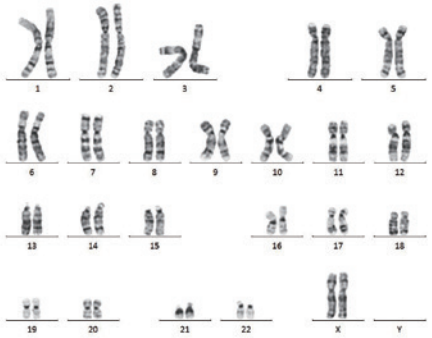
Cell expansion after thawing



Cell stocks of 1210B2 hiPSC were prepared with StemFit (red bar), Medium A (blue bar), or Medium B (green bar). Cell stocks were thawed with each respective medium and cultured for a week. Cell stocks prepared with StemFit showed superior cell growth compared to Medium A or Medium B. The superior cell growth of StemFit allows the expansion of the cell stocks with greater efficiency.

Karyotype analysis after 52 passages



201B7 cells were passaged in StemFit medium for 52 passages. G-band staining showed cells maintained a normal karyotype after 52 passages.

Eat Well, Live Well.
AJINOMOTO

For further information, please contact
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AJINOMOTO CO., INC. AminoScience Division
15-1, Kyobashi 1-Chome, Chuo-Ku, Tokyo 104-8315, Japan

Feeder-free medium for ES/iPS cells

StemFit Technical tips-3

Key Points for ***making cell stocks*** with ***single-cell passaging method***

Benefit 1

Highly efficient cell stock production
with a single-cell passaging protocol

Benefit 2

Superior cell growth after thawing

Eat Well, Live Well.
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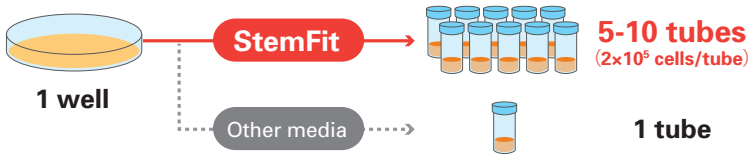
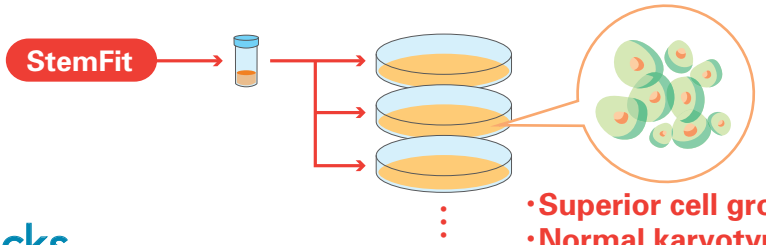
StemFit Technical tips-3

Highly efficient cell stock production



Excellent cell growth and stable karyotype



● Efficient protocol for making cell stocks

Freezing

Freezing Protocol

Procedure (6-well plate)

< Cell preparation >

1. Culture cells with StemFit for more than 2 passages.
2. Use 50-80% confluent cells for cell stocks.

< Procedure >

1. Detach and count cells.
2. Aspirate the medium and resuspended the cells at 1 x 10^6 cells/mL in STEM-CELLBANKER® freeze solution. (Bambanker® can be used as well)
3. Dispense 200 µL (2 × 10^5 cells) per cryovial.
4. Cool using a programmed freezer (-1˚C/min), or in a Freezing Container (e.g. CoolCell® or Mr. Frosty™) at -80˚C for at least 3 hrs.
5. Transfer to liquid nitrogen within a few days for extended storage.

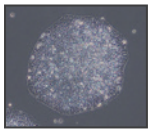
Aspirate the medium and wash once with 2 mL of PBS

Add 500 µl/well of Accutase and incubate at 37 °C for 10 min

* TrypLE™ can also be used for cell dissociation

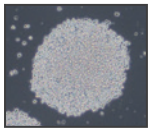
* Incubation time may vary depending on the matrix

・**Before incubation with Accutase**

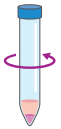


・**Gaps in the colonies appear and dissociation of colonies is apparent**

Whole colony can be smoothly detached



Count the cells and centrifuge the tubes (300 X g) at room temperature



Thawing

Thawing Protocol

Procedure (6-well plate)

1. Thaw frozen stock immediately in a water bath at 37°C for ~ 1 min until only small ice particles remain.
2. Transfer the cell suspension to 5 mL of culture media with gentle pipetting (1-2 times).
3. Spin the cells at 300 x g, RT, for 4 min.
4. Resuspend in 1 mL of medium and count cells.
5. Seed 65,000 live cells to 1.5ml culture media containing 10 µM Y-27632.
6. After > 24hr, change to regular culture medium (NOT containing Y-27632).