HyCell-STEM-FF protocol: stem cell expansion and maintenance in feeder-free culture systems

HyClone media and supplements

Product description

HyCell™-STEM-FF medium is designed for culturing human embryonic stem cells (hESCs) and induced pluripotent stem (hiPS) cells under feeder-free culture conditions. This defined, serum-free formulation is used for expanding and maintaining stem cell colonies with daily feeds. HyCell-STEM-FF is supplied as a 6× supplement that is added to Dulbecco's modified eagle medium (DMEM)/F12 basal medium to create a complete medium. Basic fibroblast growth factor is added to the completed medium at time of use.

Required materials

- HyClone™ HyCell-STEM-FF medium kit (SR30004.KT), including
  - HyClone HyCell-STEM-FF 6× supplement, 100 mL (SR30004.01)
  - HyClone DMEM/F12 with L-glutamine and HEPES, 500 mL (SH30023.01)
- Vitronectin (recombinant human full-length vitronectin)
- Basic fibroblast growth factor (bFGF)
- HyClone AdvanceSTEM™ Dulbecco's phosphate buffered saline (DPBS) without Calcium and Magnesium (SH30850.02)
- Cell harvesting solution (0.5 mM EDTA)
- Culture plates as recommended by vitronectin supplier
- Optional: Y-27632 ROCK inhibitor

Storage and handling

Upon receipt, store HyCell-STEM-FF 6× supplement frozen (-20°C or lower) and DMEM/F12 at 2°C to 8°C. Before use, thaw 6× supplement overnight at 2°C to 8°C. Avoid repeated freezing and thawing of supplement.

Protocol

Preparing complete medium

Step	Action
1	Add the thawed 100 mL bottle of 6× supplement to the 500 mL DMEM/F12 basal medium bottle.
2	Gently invert the bottle of medium to mix. • Filter the medium if desired using a surfactantfree cellulose acetate filter. If maintaining aseptic conditions, the medium does not need to be filtered.
3	The complete HyCell-STEM-FF medium can be stored at 2°C to 8°C, where it is stable for four weeks.
4	Aliquot volume of medium needed for daily use and warm in a 37°C water bath for 10 to 20 min. Warming the entire bottle of complete medium is not recommended. Do not leave medium in the water bath for an extended period of time. Maintain aseptic conditions.
5	Add 10 ng/mL basic fibroblast growth factor (bFGF) to the growth medium at time of use and mix by gentle swirling.

Thawing stem cells into HyCell-STEM-FF

Step	Action
1	Prior to thawing hESC or hiPS cells, prepare vitronectincoated plates according to manufacturer's instructions.
2	Quickly thaw cells in a 37°C water bath. Remove the cryovial from the water bath before the ice in the vial has completely melted.
3	Spray the vial with 70% ethanol and transfer to a biological safety cabinet.
4	Transfer the cells drop-wise from the cryovial to the warm HyCell-STEM-FF medium in a conical tube while swirling. Use warm growth medium to rinse and thaw any remaining ice left in the vial. Gently mix the cell suspension. Suggestion: Use 5 mL of complete medium for every 1 mL of cryopreserved cell solution. Cells can be directly thawed into HyCell-STEM-FF. No adaptation to the medium is necessary.
5	Centrifuge at 200 × g for 5 min to pellet cells. Aspirate supernatant.
6	When ready to plate cells, add 10 ng/mL of bFGF to the HyCell-STEM-FF medium. Optional: 10 µM Y-27632 may be used in the growth medium during the first ~ 48 h after thawing depending on the cell type.

Step	Action
7	Resuspend cells in warm HyCell-STEM-FF medium containing 10 ng/mL bFGF.
8	Add cell suspension to plate using 4.5 mL for one well of a 6-well plate, and distribute cell suspension evenly in the well by moving the plate in a figure-8 pattern.
9	Incubate cells at 37°C in 5% CO_2. Do not change the medium for the first 48 h.
10	Approximately 48 h post seeding, change the medium with HyCell-STEM-FF medium containing freshly added 10 ng/mL of bFGF. Use 3 mL/well for 6-well plates.
11	Change medium daily until the cells are ready to be passaged (typically, four to five days between each passage). Passage cells prior to colonies touching each other. ***Note:*** *Cells in HyCell-STEM-FF recover quickly from a thaw and might need to be split sooner than expected. Monitor your cell line closely when using HyCell-STEM-FF for the first time.*

Passaging cells with 0.5 mM EDTA

Step	Action
1	Prior to passaging the hES or hiPS cells, prepare vitronectin-coated plates according to manufacturer's instructions.
2	Rinse wells with DPBS.
3	Add 1 mL of 0.5 mM EDTA to each well of a 6-well plate. Incubate for 4 to 8 min at 37°C. Incubation time will vary per cell line. Monitor cells at 5 min. Scale volume of Ethylenediaminetetraacetic acid (EDTA) based on surface area.
4	Once cells round up, remove the cell suspension from the plate and add 5 mL of HyCell-STEM-FF. Do not add medium directly to the plate as the cells will start to reattach.
5	Centrifuge at 200 × g for 5 min to pellet cells. Aspirate supernatant.
6	When ready to plate, add 10 ng/mL of bFGF to the HyCell-STEM-FF medium. Optional: 10 µM Y-27632 may be used in the growth medium during the first ~ 48 h after splitting depending on the cell type.
7	Resuspend cells in warm HyCell-STEM-FF medium containing 10 ng/mL bFGF. Add cell suspension to the plate using 4.5 mL for one well of a 6-well plate. Seeding densities can range from 7500 to 20 000 cells/well in a 6-well plate. ***Note:*** *The cell seeding density might need to be adjusted according to your cell type. Successful seeding densities can be lower than expected. If using split ratios for plating rather than counting cells, empirically determine the appropriate split ratio. Split ratios may need to be as low as 1:40 or 1:100. If using different size culture ware, adjust cell seeding densities and volumes according to surface area. Split cultures every four to five days. Do not allow cultures to be grown past five days. To limit spontaneous differentiation, split cells prior to individual colonies are touching each other.*
8	Evenly distribute the cell suspension in the well by moving the plate in a figure-8 pattern.
9	Incubate cells at 37°C in 5% CO_2. Do not change the medium for the first 48 h.
10	Approximately 48 h post seeding, change the medium with HyCell-STEM-FF medium containing freshly added 10 ng/mL of bFGF. Use 3 mL/well for 6-well plates.
11	Change medium daily until the cells are ready to be passaged (typically, four to five days between each passage). Passage cells prior to colonies are touching each other. ***Note:*** *Cells in HyCell-STEM-FF often grow rapidly and may need to be split sooner than expected. Monitor your cell line closely when using HyCell-STEM-FF for the first time.*

Adapting stem cells lines to HyCell-STEM-FF

Step	Action
1	Cultures can be transitioned into HyCell-STEM-FF with no adaptation required for any media used for the cells during passage or upon thaw.

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