Stem cell expansion and maintenance with feeder culture systems

HyClone media and supplements

Overview

HyCell™-STEM supplement is designed for culturing human embryonic stem cells (hESCs) and induced pluripotent stem (hiPS) cells with mouse embryonic fibroblast (MEF) feeder cells. This defined, serum-free formulation is used for expanding and maintaining stem cell colonies with daily or every other day feeds. HyCell-STEM is provided as a 6× supplement that is added to DMEM/F12 to create a complete medium. Basic fibroblast growth factor is added to the completed medium at time of use.

Material supplied

- HyCell-STEM media kit (SR30003.KT)
- HyClone™ HyCell-STEM 6× supplement, 100mL
- HyClone DMEM/F12 with L-glutamine and HEPES, 500mL
- HyCell-STEM supplement only (SR30003.01)
- HyClone HyCell-STEM 6× supplement, 100mL

Materials required, not supplied

- DMEM/F12 with L-glutamine and HEPES, 500mL (if not purchased with a kit)
- Mouse embryonic fibroblast (MEF) feeder cells
- Basic fibroblast growth factor (bFGF)
- Cell harvesting solution or manual tool for dissociation
- Tissue culture plates and consumables
- Optional: ROCK inhibitor Y-27632 (Sigma Aldrich)

Storage and handling

Upon receipt, store HyCell-STEM 6× supplement frozen (-20°C or below) 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 the supplement.

Protocol

Preparing a complete media

Step	Action
1	Add the entire thawed 100 mL bottle of 6× supplement to the 500 mL DMEM/F12 basal medium.
2	Gently invert the medium bottle to mix. • Filter the medium if desired using a surfactant-free cellulose acetate filter. If aseptic conditions are maintained, the medium does not need to be filtered.
3	Store complete HyCell-STEM medium refrigerated. • The complete growth medium is stable at 2°C to 8°C for 4 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. Prepare only the volume of medium needed for daily use. • Do not leave medium in the water bath for extended periods of time. Maintain aseptic conditions.
5	Add 10 ng/mL bFGF to the growth medium at time of use and mix by gentle swirling.

Thawing stem cells into HyCell-STEM

Step	Action
1	Prepare MEF feeder cell layer at least 24 hours prior to thawing hES/hiPS cells.
2	Quickly thaw hES/hiPS cells in a 37°C water bath, removing 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 with swirling from the cryovial to warm HyCell-STEM medium in a conical tube. 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. No adaptation to the medium is necessary.
5	Centrifuge at 200 x *g* for 5 min to pellet the cells. Aspirate supernatant.

Step	Action
6	When ready to plate cells, add 10 ng/mL of bFGF to the HyCell-STEM medium. • 10 µM ROCK inhibitor may also be added to the growth medium during the first ~ 48 hours after thawing depending on the cell type.
7	Resuspend cells in warm HyCell-STEM medium containing 10 ng/mL bFGF. Rinse the MEF cells with warmed basal DMEM/F12 to remove the MEF cell medium.
8	Add hES/hiPS cell suspension to the plate of MEF cells: use 4.5 mL for one well of a 6-well plate. Distribute the cells suspension evenly in the well by moving the plate in a figure-8 pattern.
9	Incubate cells at 37°C with 5% CO_2. Do not change the medium for the first 48 hours.
10	Approximately 48 hours post-seeding, change the medium with HyCell-STEM medium containing freshly added 10 ng/mL of bFGF. Use 3 mL/well for 6-well plates.
11	Passage cells when the cultures become approximately 80% confluent and before the colonies begin to touch. Passage cells as you normally do with enzymes or manual dissociation. • Cells in HyCell-STEM recover quickly from a thaw and might need to be split sooner than expected. Monitor your cell line closely when using HyCell-STEM for the first time.

Feeding and passaging cells

Step	Action
1	Feed cells daily or every other day by removing the spent medium from the culture and replacing with HyCell-STEM medium containing freshly added 10 ng/ml of bFGF. Use 3 mL/well for 6-well plates. • ES and MEF cells grown in HyCell-STEM might differ in morphology from other growth media and supplements. ES colonies can appear to be flatter. • If working with a robust cell line, medium can be changed every other day. If a medium change (feeding) falls on a Friday, add double the amount of medium (6 mL/well for 6-well plates). Cultures thus fed do not need to be tended until Monday. • To limit spontaneous differentiation, split cells prior to individual colonies touching. Adjust seeding density to yield desired growth characteristics. • Recommended split schedule when feeding every other day and splitting weekly: - Monday split; Wednesday feed; Friday double feed; Monday split - Tuesday split; Friday double feed; Monday feed; Tuesday split - Wednesday split; Friday double feed; Monday feed; Wednesday split - Thursday split; Saturday feed; Monday feed; Wednesday feed; Thursday split - Friday split; Monday feed; Wednesday feed; Friday split
2	Dissociate cells with enzymes (HyQTase™ solution) or manual dissociation following manufacturer's recommendations.
3	Resuspend cells in warm HyCell-STEM medium containing 10 ng/mL bFGF. • 10 µM ROCK inhibitor may also be added to the growth medium during the first ~ 48 hours after splitting depending on the cell type.
4	Rinse the MEFs with warmed basal DMEM/F12 to remove the MEF medium.
5	Add cell suspension to the plate of feeder cells using 4.5 mL for one well of a 6-well plate. Seeding densities can range from 6000 to 24 000 cells/well in 6-well plates. At these seeding densities, cultures can usually be split weekly. • Adjust seeding density according to harvest method, length of time in culture, and cell line. Successful seeding densities might 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 areas.
6	Evenly distribute the cell suspension in the well by moving the plate in a figure-8 pattern.
7	Incubate cells at 37°C with 5% CO_2. Do not change the medium for the first 48 hours after seeding.
8	Approximately 48 hours post-seeding, change the medium using 3 mL/well for 6-well plates with HyCell- STEM medium containing freshly added 10 ng/mL of bFGF.
9	Follow split and feeding schedule described in step 1 above.

Adapting stem cells to HyCell-STEM

Step	Action
1	Stem cell cultures can be transitioned into HyCell-STEM medium with no adaptation during passage or upon thaw.

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