Stemmera

Human ESCs/iPSCs Xeno-Free Medium (hStemXFM)

Catalog Number: ST02002
Size: 500 ml

Xeno-Free Medium (XFM) is a product that contains no animal- and human-derived component. It supports cell growth in the absence of any animal pathogens during human stem cell culturing, including ESCs and iPSCs.

Product Description

Stemmera™ Human ESCs/iPSCs Xeno-Free Medium (hStemXFM) is a ready-to-use and fully defined animal-free culture medium. It is formulated for maintenance of human induced Pluripotent Stem Cells (hiPSCs) and human Embryonic Stem Cells (hESCs), which is intended for use in clinical based research and drug screening. It also can be used in basic research.

Product Component

Component	Size	Storage	Cat #
Basal Medium with L-glutamine and Sodium Bicarbonate	500 ml	2°C to 8°C	ST02002-BM
XFM Supplement	3.5 ml	-20°C	ST02002-S1

Mixture of two components is a ready-to-use complete medium.

Storage and Handling

Stemmera™ hStemXFM is shipped separately with blue ice pack for Basal Medium, while the XFM Supplement is shipped with dry ice. Store Basal Medium at 2-8°C and XFM Supplement at -20°C upon arrival and until the expiration dates on the product label. Avoid multiple freeze/thaw cycles. The complete medium should remain stable for up to a month when stored at 2-8°C in the dark. Make an aliquot of complete medium for daily use, avoid warm up cycles. Protect from light is preferred.

Product Use

This product is intended for *in vitro* use and research use only. Not intended for human or animal diagnostic or therapeutic uses.

General Precautions

1. Use aerosol barrier tips. Change tips after each use.
2. Always use fresh, clean gloves and wear lab coats.
3. Material Safety Data Sheet (MSDS) is available online.
4. Clean working space with 70% ethanol or other suitable disinfectant.

Culture Conditions

Media: Stemmera™ hStemXFM

Cell: human induced Pluripotent Stem Cells (hiPSCs) and human Embryonic Stem Cells (hESCs)

Culture type: Single cell passaging and adherent culture

Temperature range: 37°C

Incubator atmosphere: Humidified atmosphere at 5% CO_2 and/or 5% O_2. Ensure proper gas exchange and minimize exposure

Recommended culture vessels: rLaminin (Corning, Cat # 354220) coated plate, dish or flask. Adjust volume of cell number according to vessel sizes.

Protocol

- **Preparation of Complete XFM**

  Add 3.5 ml of frozen XFM Supplement (Cat # ST02002-S1) in 500 ml of Basal Medium (Cat # ST02002-BM), mix well and filter with 0.22µm filter.
- **Aliquot and Pre-warm Complete XFM** at room temperature for daily use.
- **Coat the vessels with Corning rLaminin**

  Use Corning rLaminin (Cat # 354220) to coat the vessels and incubate in a 37°C, 5% CO_2 incubator for at least 30 minutes or overnight. The coated vessels can be used within one week. Prior to use, remove all rLaminin immediately and replace with pre-warmed complete Stemmera™ hStemXFM.

Note: Complete Stemmera™ hStemXFM is stable for up to 4 weeks when stored in the dark at 2°C to 8°C within the expiration dates of all components.

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Recovery of hiPSCs and hESCs in complete hStemXFM

1. Rapidly thaw one vial of frozen human Stem Cells in 37°C water bath.
2. Gently pipet the entire contents of the cryovial into a sterile 15 ml conical tube with complete medium.
3. Centrifuge tube at 1000 rpm for 5 minutes.
4. Aspirate and discard supernatant. Take extreme care not to disturb cell pellet.
5. Re-suspend the cells in 1 ml of pre-warmed complete culture medium with Rock inhibitor Y27632 at 10 µM (Fisher Scientific, Cat # 50-863-7).
6. Transfer the cells into rLaminin pre-coated 6-well plate and add sufficient complete medium to the wells (2 ml of medium per well).
7. Incubate the cells in complete XFM with Rock inhibitor in a 37°C, 5% CO_2 and/or 5% O_2 (Low oxygen) incubator overnight.
8. Change medium to complete XFM without Rock inhibitor on the following day and change medium daily until the cells have reached 80-90% confluency and subculture the cells.

Subculturing stem cells in complete Stemmera™ hStemXFM

9. When cells reach 80-90% confluency, aspirate spent medium from the wells and discard.
10. Add 1 ml of Stemmera™ Non-Enzymatic Cell Dissociation Solution (Cat # ST03001) to each well. Ensure the solution covers the cell monolayer. Incubate for 5 minutes in a 37°C, 5% CO_2 and/or 5% O_2 (Low oxygen incubator) incubator.
11. Periodically observe cells under an inverted microscope, until the cells begin to round up. (Note: Avoid the cells from detaching completely, check below *Troubleshooting*).
12. Gently aspirate and discard the Cell Dissociation Solution from the wells (Prevent the cells from drying up).
13. Add 1 ml of complete XFM in each well and use a 1 ml pipette to gently pipet cells up and down a few times in well to further break up cell colonies until getting the single cells. (Note: Pipet carefully to reduce foaming).
14. Transfer the cells into a new well of pre-coated 6-well plate and add sufficient complete medium with Rock inhibitor (2 ml of XFM each well of 6-well plate, the split ratio is 1:6 – 1:12)
15. Incubate the cells in a 37°C, 5% CO_2 and/or 5% O_2 (Low oxygen) incubator overnight.
16. Change medium to complete XFM without Rock inhibitor on the following day and change medium daily until the cells have reached 80-90% confluency and subculture the cells or cryopreserve cells in liquid nitrogen for long-term storage.

Troubleshooting:

- *Incubation time in cell dissociation solution is dependent on the cell's density and cell numbers.*
- *If the cells were detached, add same volume of culture medium and resuspend the cells, collect in 15 ml of conical tube, centrifuge the cell and plate the cells.*

Cryopreserve stem cells in Stemmera™ Serum-Free Cryopreservation Solution

17. Change medium 1-2 hours prior to freezing the cells.
18. Harvest cells by following steps 9 through 13 in **Subculturing stem cells in complete Stemmera™ hStemXFM.**
19. Transfer 1 ml of single cell suspension into 15 ml conical tube.
20. Centrifuge tube at 1000 rpm for 5 minutes.
21. Aspirate and discard supernatant taking extreme care not to disturb cell pellet.
22. Calculate the volume of cryopreservation solution required to give a cell density of $2x10^6$ viable cells/ ml.
23. Re-suspend the pellet with Stemmera™ Serum-Free Cryopreservation Solution (Cat # ST03002) at the accurate volume and aliquot the cells to cryovials (1 ml/vial).
24. Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (1^oC decrease per minute).
25. Transfer frozen cells to the vapor phase of liquid nitrogen. We recommend vapor phase storage at -200^oC to -150^oC for a few years.

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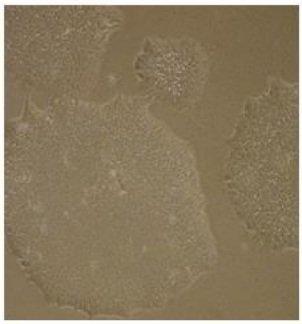
Quality Control

This product is used to maintain the pluripotency of human ESCs/iPSCs under xeno-free culture condition that is without animal pathogen contamination. To ensure the quality, the following images represents the pluripotent morphology of human ESCs/iPSCs at passages 25 growing on rLaminin with Stemmera™ hStemXFM.



Human iPS Colonies at passage 25 growing with Stemmera XFM

Warranty and Limited Liability

Stemmera™ will not be liable for any damage caused by misuse, improper handling and storage of the product, non-compliance with precautions and procedures, and damages caused by events occurring after the product is released.

Related Products

Product	Cat #	Size
Alkaline Phosphatase Detection Kit (Ready-to-Use) - Blue	ST01001	50 tests
Alkaline Phosphatase Detection Kit (Ready-to-Use) - Red	ST01002	50 tests
Human ESCs/iPSCs Serum-/Feeder-Free Medium (hStemSFM)	ST02001	500 ml
Non-Enzymatic Cell Dissociation Solution (1X)	ST03001	100 ml
Serum-Free Cryopreservation Solution for Human ESCs/iPSCs	ST03002	50 ml

Technical Support

For more product and technical information, please refer to www.stemmera.com.

For further assistance, email your inquiries to our Technical Support team at techsupport@stemmera.com.

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