Data Sheet

LX-2 Hepatic Stellate Cell Line-SF Adapted

SCC064SF

Pack Size: ≥ 1x10^6 viable cells/vial

Store in liquid nitrogen.

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for human or animal consumption.

Background

Hepatic stellate cells (HSCs) play a crucial role in the progression of liver fibrosis, a condition characterized by excessive extracellular matrix deposition and liver scarring. LX-2 cells are immortalized human hepatic stellate cells that retain key features of primary HSCs, making them an invaluable tool for studying liver pathology.[1] These cells were generated through the immortalization of primary human hepatic stellate cells using the SV40 large T antigen, followed by selective culture under low serum conditions.[2] LX-2 cells retain the functional characteristics of primary HSCs, enabling researchers to investigate their role in liver disease effectively.

The unique advantage of LX-2 cells is their viability in serum-free media, which facilitates more controlled experimental conditions and reduces variability associated with serum components. LX-2 Hepatic Stellate Cell Line-Serum-Free (SF) adapted cells (SCC064SF), are specifically adapted to grow and propagate in the specialized serum-free medium, LX-2 Human Hepatic Stellate Serum-Free Media (SCM064SFM). This adaptation addresses common issues of culture variability and provides a stable population of human hepatic stellate cells that are homogeneous in nature. SCC064SF cells have been extensively characterized, demonstrating that they retain the key features and functionality of LX-2 cells grown in serum-containing media. This makes them particularly suitable for culture-based studies of human hepatic fibrosis, as they allow for more reproducible and reliable results.

Furthermore, the use of serum-free conditions not only enhances the consistency of experimental outcomes but also minimizes the potential for serum-derived factors to influence cellular behavior. This is especially important in studies aimed at elucidating the mechanisms underlying liver fibrosis and evaluating therapeutic interventions. The SCC064SF cell line represents a significant advancement in the study of hepatic stellate cells, providing a robust platform for investigating the pathophysiology of liver diseases and the development of novel treatment strategies.

Source

Human hepatic stellate primary cells were isolated from a consenting normal human donor following established protocols outlined by Friedman et al.[2]

Short Tandem Repeat

D3S1358:	13, 15	D8S1179:	13	D13S317:	11, 13	CSF1PO:	10, 12
D7S820:	11	D21S11:	28, 31	D16S539:	13	AMEL:	X, Y
vWA:	17	D18S51:	12	TH01:	9.3	Penta D:	13, 15
FGA:	21, 26	D5S818:	11, 12	TPOX:	8, 9	Penta E:	5, 21

Quality Control Testing

- SCC064SF, LX-2 Hepatic Stellate Cell Line-Serum-Free (SF) adapted cells are verified to be of human origin and negative for rat, Chinese hamster, Golden Syrian hamster, and nonhuman primate interspecies contamination, as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells tested negative for infectious diseases against a Human Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

Storage and Handling

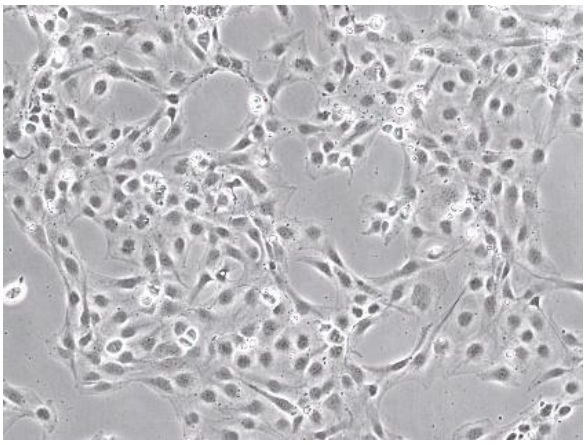
LX-2 Hepatic Stellate Cell Line-Serum-Free adapted cells should be stored in liquid nitrogen until use. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Materials Required (Not provided)

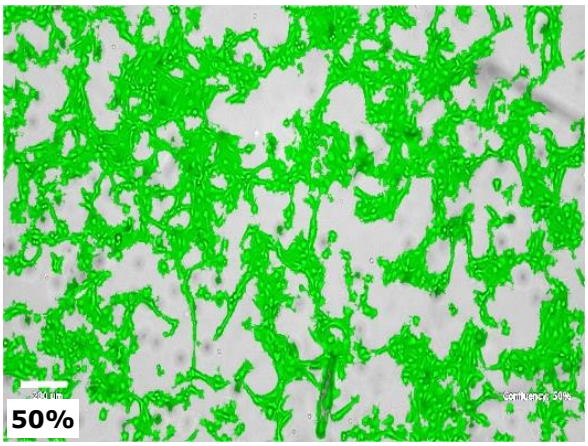
- LX-2 Human Hepatic Stellate Serum-Free Media-SCM064SFM
- Accutase®-SCR005
- D-PBS-D5652
- CryoStor10-C2874

Representative Data

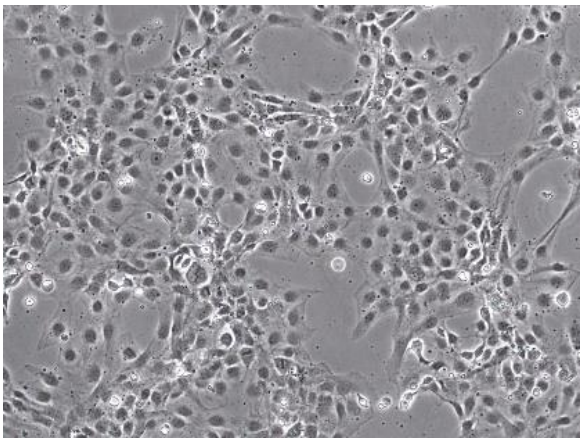
A



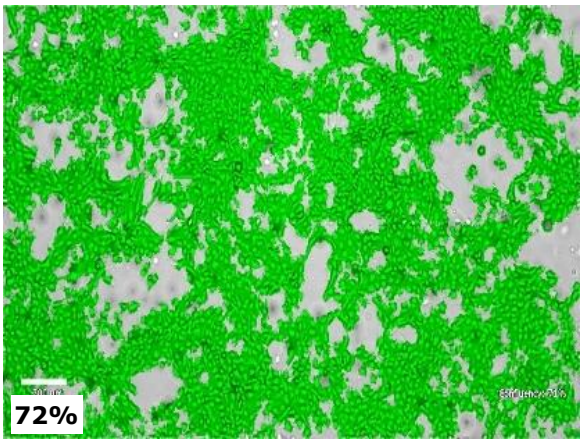
B



C



D



E

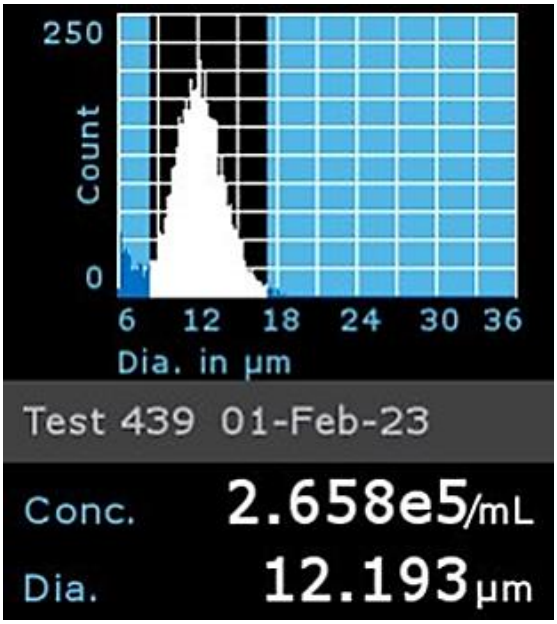


Figure 1: 1 vial of LX-2 Hepatic Stellate Cell Line-Serum-Free (SF) adapted cells, SCC064SF, cells was thawed in a T75 flask and cultured in SCM064SFM (LX-2 Human Hepatic Stellate Serum-Free Media). Images represent 10x phase contrast and confluency (measured using Millicell® Digital Cell Imager-MDCI10000) images on day 5 (**A**, **B**), day 7 (**C**, **D**) and cell count measured using Scepter™ 3 (**E**).

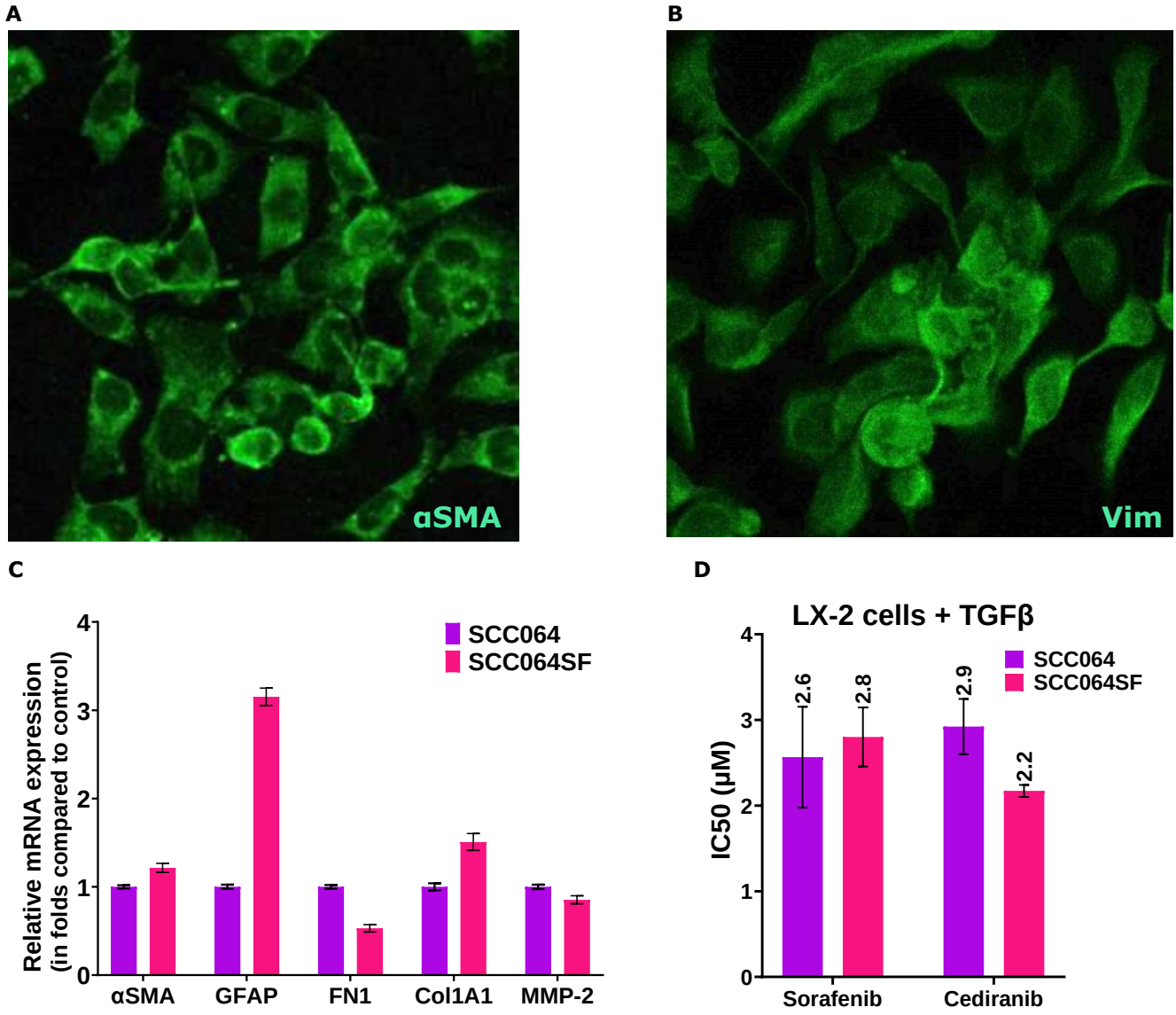


Figure 2: (**A**, **B**) LX-2 Serum-free (SF) adapted cells, SCC064SF, expressed alpha smooth muscle actin (αSMA) and vimentin (Vim) as analyzed by immunofluorescence. (**C**) Real-time quantitative PCR data showing fold changes in expression of key markers in SCC064SF cells as compared to LX-2 cells, SCC064, grown in 2% fetal bovine serum containing medium. LX-2 cells (SCC064) cultured in 2% FBS DMEM and LX-2 Serum-free (SF) SCC064SF cultured in LX-2 Human Hepatic Stellate Serum-Free Media (SCM064SFM) were activated with TGF-β and were treated with various concentrations of Sorafenib and Cediranib. (**D**) IC50 values were determined. Our data indicated that the IC50 did not vary significantly between SCC064 and SCC064SF for both the drugs tested.

Protocols

Thawing of Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells are thawed in warm, equilibrated LX-2 Human Hepatic Stellate Serum-Free Media (SCM064SFM) containing 1X Pen/Strep (P4333).
2. Remove the vial of LX-2 Hepatic Stellate Cell Line-Serum-Free (SF) adapted cells (SCC064SF) from liquid nitrogen and incubate in a 37 °C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

   IMPORTANT: Do not vortex the cells.
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1-2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of SCM064SFM (Step 1 above, pre-warmed to 37 °C) to the 15 mL conical tube.

   IMPORTANT: Do not add the whole volume of media at once to the cells. This may result in decreased cell viability due to osmotic shock.
6. Gently mix the cell suspension by slowly pipetting the cells up and down twice.

   IMPORTANT: Do not vortex the cells.
7. Centrifuge the tube at 150 x *g* for 4-5 minutes to pellet the cells.

   IMPORTANT: Do not use high centrifugal speed/force.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative.
9. Resuspend the cells in 1 mL of SCM064SFM (Step 1 above, pre-warmed to 37 °C).
10. Count cells using hemacytometer.
11. Add the cells gently into a T75 flask containing 17 mL of SCM064SFM medium (Step 1, pre-warmed to 37 °C).
12. Incubate at 37 °C in a 5% CO_2 humidified incubator.
13. Exchange the medium with fresh medium on day 3 and then once every 2-3 days.
14. Monitor cell confluency using the Millicell® Digital Cell Imager (MDCI10000).
15. When the cells are approximately 75%-85% confluent (7-8 days after plating cells) they can be dissociated with Accutase® (SCR005) and passaged or alternatively frozen for later use.

Subculturing of Cells

1. Carefully remove the medium from the T75 tissue culture flask containing the confluent layer of LX-2 Serum-free (SF) adapted cells (SCC064SF).
2. Wash the cells by adding 5 mL of D-PBS gently. Carefully remove the D-PBS.
3. Add 5 mL of Accutase® and incubate in a 37 °C incubator for 1-3 minutes.
4. Inspect the plate and ensure the complete detachment of cells by gently tapping the side of the plate with the palm of your hand.
5. Add 8 mL of LX-2 Human Hepatic Stellate Serum-Free Media (SCM064SFM) (pre-warmed to 37 °C) to the plate.
6. Gently rotate the plate to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
7. Centrifuge the tube at 150 x *g* for 5 minutes to pellet the cells. Discard the supernatant.
8. Apply 2 mL of SCM064SFM (pre-warmed to 37 °C) to the conical tube and resuspend the cells thoroughly.

   IMPORTANT: Do not vortex the cells.
9. Count the number of cells using a hemocytometer.
10. Plate the cells to the desired density (typical split ratio is 1:3).

Cryopreservation of Cells

SCC064SF, LX-2 serum-free (SF) adapted cells grown in LX-2 Human Hepatic Stellate Serum-Free Media (SCM064SFM) can be frozen in Cryostor10 (C2874) by storing overnight at -80 °C in Nalgene® slow freeze Mr. Frosty® container and then transferring to liquid nitrogen subsequently.

Sequential adaptation to serum free media protocol

1. LX-2 cells (SCC064) are thawed in DMEM High Glucose Medium (SLM-021-B) containing 10% FBS (ES-009-B), 1X Pen/Strep (TMS-AB2-C) and 1X Glutamine (TMS-002-C).
2. Once thawed, cells are expanded in 2% FBS supplemented media using the same components listed above.
3. When cells are around 85% confluent, change the medium to LX-2 Human Hepatic Stellate Serum-Free Media (SCM064SFM).
4. Allow cells to grow in SCM064SFM for a minimum of 1 day before passage.
5. Passage cells at 1:2 ratio when cells are ~90% confluent in SCM064SFM. This is designated as SFMp1.
6. Allow cells to grow and when cells are ~90% confluent, passage at 1:2 split ratio into SCM064SFM. This is designated as SFMp2.
7. Continue the above for two more passages, maintaining a 1:2 split ratio. Cells will proliferate slowly until SFMp3.
8. By SFMp4 and SFMp5, cells will have adapted to the serum-free medium condition and can be passaged on Days 6-7 (at 85-90% confluency) at 1:3 split ratio.

   Note: Cells may grow as patches and may not form a complete monolayer during the first two passages of adaptation. Monitor cells closely to avoid them becoming more densely packed during this time.

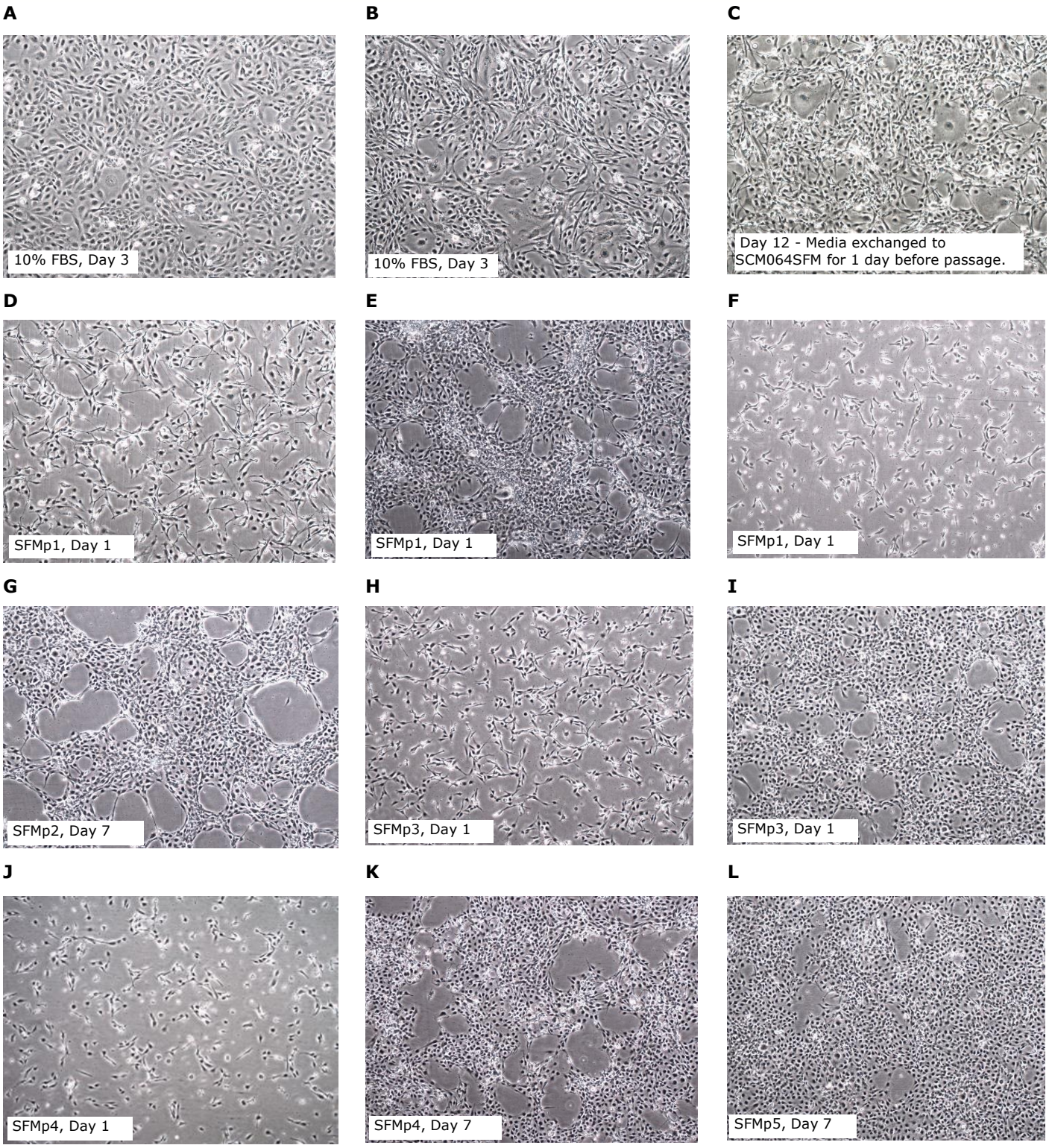


Figure 3: Adaptation of LX-2 cells from medium containing 10% FBS to serum-free medium, LX-2 Human Hepatic Stellate Serum-Free Media (SCM064SFM). 4X images taken at each passage, showing cell confluency ranging from 85%-90% during the adaptation process. Each image is labeled with SFMp to indicate the number of passages in SCM064SFM and "Day" to indicate the day of passage (For example, SFMp5=5 passages in serum-free medium).

LX-2 cells typically take 7 passages to adapt completely to serum-free medium. During the initial phases of adaption (up to SFMp3), the cells take longer to reach the desired 85-90% confluency. In these initial phases, it is recommended to passage cells at 1:2 split ratio. From SFMp4 onwards, cells can be passaged at 1:3 split ratio.

Related Products

- SCM064SFM, LX-2 Human Hepatic Stellate Serum-Free Media
- SCC064, LX-2 Human Hepatic Stellate Cell Line

References

1. Xu L, Hui AY, Albanis E, Arthur MJ, O'Byrne SM, Blaner WS, Mukherjee P, Friedman SL, Eng FJ. 2005. Human hepatic stellate cell lines, LX-1 and LX-2: new tools for analysis of hepatic fibrosis. Gut 54 (1):142–151.
2. Friedman SL, Rockey DC, McGuire RF, Maher JJ, Boyles JK, Yamasaki G. 1992. Isolated hepatic lipocytes and Kupffer cells from normal human liver: morphological and functional characteristics in primary culture. Hepatology 15(2): 234-243.

Academic Use Agreement

Subject to local law

THIS PRODUCT MAY ONLY BE USED BY INDIVIDUALS EMPLOYED BY AN ACADEMIC INSTITUTION AND IS INTENDED SOLELY TO BE USED FOR ACADEMIC RESEARCH, WHICH IS FURTHER DEFINED BELOW. BY OPENING THIS PRODUCT, YOU ("PURCHASER") HEREBY REPRESENT THAT YOU HAVE THE RIGHT AND AUTHORITY TO LEGALLY BIND YOURSELF AND/OR YOUR EMPLOYER INSTITUTION, AS APPLICABLE, AND CONSENT TO BE LEGALLY BOUND BY THE TERMS OF THIS ACADEMIC USE AGREEMENT. IF YOU DO NOT AGREE TO COMPLY WITH THESE TERMS, YOU MAY NOT OPEN OR USE THE PRODUCT AND YOU MUST CALL ("SELLER") CUSTOMER SERVICE (1-800-645-5476) TO ARRANGE TO RETURN THE PRODUCT FOR A REFUND.

"Product" means LX-2 Hepatic Stellate Cell Line-SF Adapted (SCC064SF).

"Academic Research" means any internal in vitro research use by individuals employed by an academic institution. Academic Research specifically excludes the following uses of whatever kind or nature:

- Re-engineering or copying the Product
- Making derivatives, modifications, or functional equivalents of the Product
- Obtaining patents or other intellectual property rights claiming use of the Product
- Using the Product in the development, testing, or manufacture of a Commercial Product
- Using the Product as a component of a Commercial Product
- Reselling or licensing the Product
- Using the Product in clinical or therapeutic applications including producing materials for clinical trials
- Administering the Product to humans
- Using the Product in collaboration with a commercial or non-academic entity

"Commercial Product" means any product intended for: (i) current or future sale; (ii) use in a fee-for-service; or (iii) any diagnostic, clinical, or therapeutic use.

Access to the Product is limited solely to those officers, employees, and students of PURCHASER's academic institution who need access to the Product to perform Academic Research. PURCHASER shall comply with all applicable laws in its use and handling of the Product and shall keep it under reasonably safe and secure conditions to prevent unauthorized use or access.

These use restrictions will remain in effect for as long as PURCHASER possesses the Product.

COMMERCIAL OR NON-ACADEMIC ENTITIES INTERESTED IN PURCHASING OR USING THE PRODUCT MUST CONTACT licensing@emdmillipore.com AND AGREE TO SEPARATE TERMS OF USE PRIOR TO USE OR PURCHASE.

Genetically Modified Organisms (GMO)

This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient des organismes génétiquement modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Document Template 20306518 Ver 6.0

23174835 Ver 1.0, Rev 14JAN2024, AV