

Data Sheet

LX-2 Human Hepatic Stellate Serum-Free Media

SCM064SFM**Pack Size 250 mL****Store at -20 °C****FOR RESEARCH USE ONLY****Not for use in diagnostic procedures. Not for Human or Animal Consumption.**

Product Overview

LX-2 Human Hepatic Stellate Serum-Free Media (SCM064SFM) is a ready to use, serum-free culture medium specifically designed for the growth and proliferation of LX-2 human hepatic stellate cells. Hepatic stellate cells are a major cell type responsible for liver fibrosis following their activation into fibrogenic myofibroblast-like cells in diseases such as chronic alcoholism, hepatitis B and C, fatty liver disease, obesity, and diabetes. LX-2 cells, immortalized human hepatic stellate cells, retain key features of primary hepatic stellate cells (HSCs) and have been extensively utilized in the study of liver fibrosis.

SCM064SFM eliminates the reliance on FBS, providing a consistent and controlled environment for cell culture. This ensures reproducibility in experimental results and reduces variability often associated with serum-based media. The media is optimized to support the growth and proliferation of LX-2 cells, enabling researchers to conduct experiments with similar cell yields and viability compared with cells grown on FBS supplemented media. By eliminating the need for FBS, SCM064SFM significantly reduces the demand for animal-derived products, contributing to ethical research practices and animal welfare.

Quality Control Testing

Appearance (Color): Clear, Red Liquid

pH: 7.2–7.8

Osmolality: 289-310 mOsm

Sterility Tested: No Growth/Pass

Endotoxin: < 2 EU/mL

Mycoplasma: Negative

Functional Assay: Thaw and culture of LX-2 cells

Storage and Handling

Upon receipt store at -20 °C. When ready to use, thaw overnight at 2-8 °C. Once thawed, mix thoroughly. Use immediately and store at 2-8 °C for up to 3-4 weeks. Multiple freeze/thaw cycles are not recommended.

Representative Data

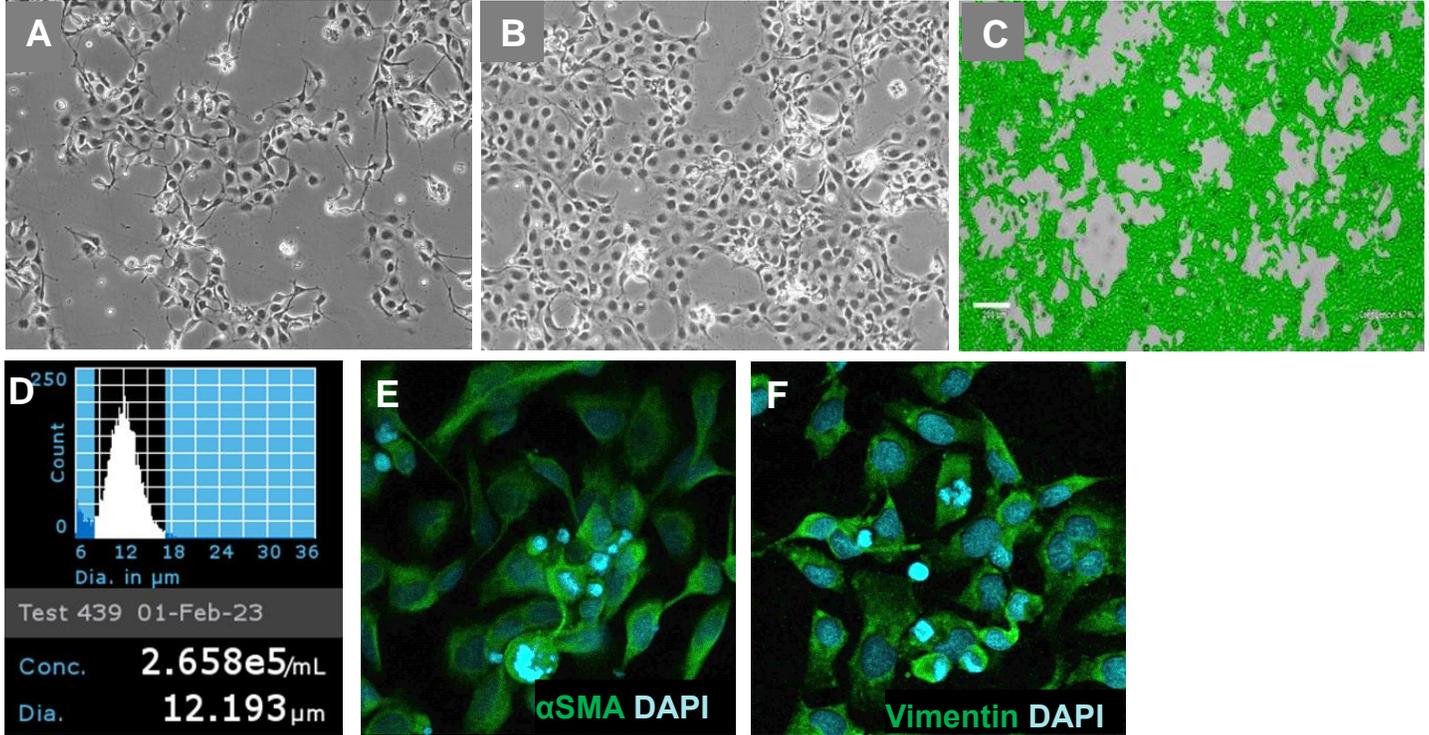


Figure 1. 2×10^6 serum-free adapted LX-2 cells cultured in SCM064SFM in a T75 flask. (A) 10X phase contrast images at day 4 (B) and day 6. (C) Cell confluency was assessed throughout the culture using the MilliCell® Digital Cell Imager (MDCI10000), (D) Cell counting was performed using the Scepter™ 3.0 handheld automated cell counter using 60 μm sensors (PHCC360KIT), (E) Cells cultured in 8-well chamber glass slides and immunostained with alpha smooth muscle actin (F) and vimentin.

Protocols

Thawing Medium

1. Thaw medium at 2-8 °C overnight or at room temperature in the dark. To minimize loss of growth factor activity, do not thaw medium at 37 °C.
2. Once thawed, mix thoroughly, and use immediately and store at 2-8 °C for up to 3-4 weeks.

Culturing LX-2 cells in SCM064SFM

Guidelines are for a T75 flask. Adjust volumes according to the culture surface area.

1. Mix thawed medium by gently swirling the unopened bottle. Do not shake or froth the medium.
2. Replace media with 18 mL SCM064SFM every 2 days.

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 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.

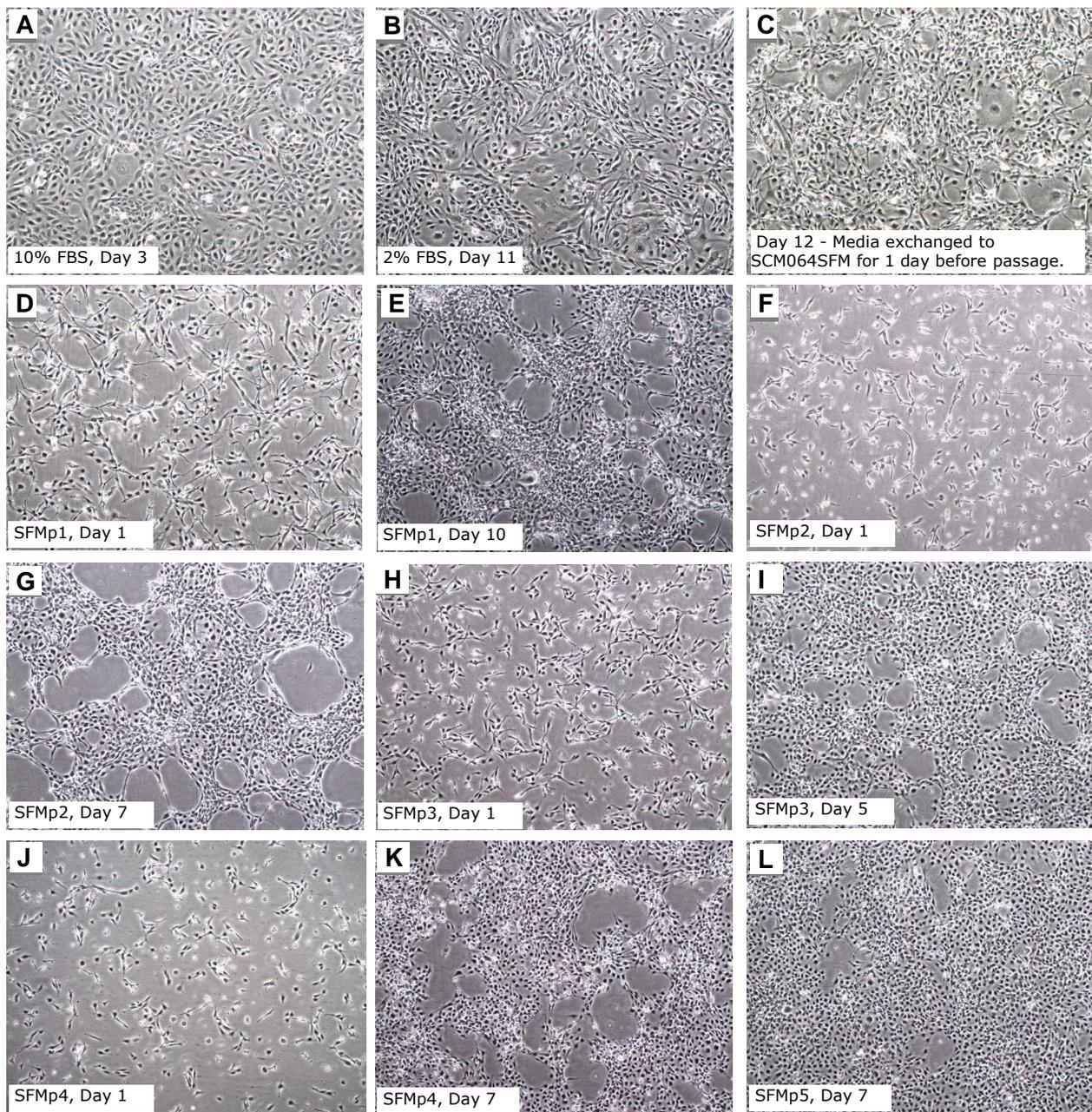


Figure 2. Adaptation of LX-2 cells from medium containing 10% FBS to serum-free medium, SCM064SFM. 4X images taken at each passage, showing cell confluency ranging from 85% - 90% during the adaptation process. Each image is labeled with SFMp to indicate the number of passages in SCM064SFM and "Day" to 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.

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