LX-2 Human Hepatic Stellate Cell Line

Immortalized Cell Line

Cat. # SCC064

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
NOT FOR HUMAN OR ANIMAL CONSUMPTION
THIS PRODUCT CONTAINS GENETICALLY MODIFIED

Pack size: ≥1X10^6 cells/vial

Store at Liquid Nitrogen

Certificate of Analysis

Background

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. There is an increasing need for renewable cell culture models that faithfully recapitulate their in vivo phenotype, particularly for human studies.

LX-2 was generated by immmortalization of primary human hepatic stellate cells with the SV40 large T antigen followed by selective culture of early passaged cells in low serum media conditions.

Immortalized LX-2 was established by Xu et al to overcome issues of culture variability and to provide a stable and unlimited source of human hepatic stellate cells that are homogeneous. These cell lines have been extensively characterized and retain key features of cytokine signaling, neuronal gene expression, retinoid metabolism, and fibrogenesis, making them highly suitable for culture based studies of human hepatic fibrosis.

Source

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

Quality Control Testing

- Each vial contains ≥ $1X10^6$ viable cells.
- Cells are tested by PCR and are negative for Hepatitis A, B, C and HIV-1 & 2 viruses.
- Cells are negatrive for mycoplasma contamination.

Storage and Handling

LX-2 cells should be stored in liquid nitrogen. The cells can be passage for at least 10 passages without significantly affecting the cell marker expression and functionality. LX-2 cells have been successfully expanded past passage 50 in culture.

References

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SPECIES LEGEND: **H** Human **Ca** Canine **M** Mouse **R** Rat **Rb** Rabbit **B** Bovine **P** Porcine **WR** Most Common Vertebrates

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Protocols

Thawing of Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on normal tissue culture ware surfaces without any additional coating.

   Cells are thawed in DMEM High Glucose (Millipore Cat. No. SLM-021-B), 10% FBS (Millipore Cat. No. ES009-B), 1X Pen/Strep (Millipore Cat. No. TMS-AB2-C) and 1X Glutamine (Millipore Cat. No. TMS-002-C) media. Onced thawed, cells are expanded in 2% FBS media using the same components listed above.
2. Remove the vial of LX-2 cells 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 or 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 9ml of 10% FBS media (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 slow pipeting up and down twice. Be careful to not introduce any bubbles.

   IMPORTANT: Do not vortex the cells.
7. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in a total volume of 10 % FBS medium (pre-warmed to 37°C).
10. Plate the cell mixture onto a T75 tissue culture flask.
11. Incubate the cells at 37°C in a 5% CO2 humidified incubator.
12. The next day, exchange the medium with fresh 2% FBS media (Step 1 above) pre-warmed to 37°C. Exchange with fresh medium every two to three days thereafter.
13. When the cells are approximately 80% confluent (3-4 days after plating cells at the density they can be dissociated with Accutase (Millipore Cat. No. SCR005) or trypsin (Millipore Cat. No. SM-2003-C) 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 cells.
2. Apply 3-5 mL of accutase or trypsin solution and incubate in a 37°C incubator for 3-5 minutes.
3. Inspect the plate and ensure the complete detachment of cells by gently tapping the side of the plate with the palm of your hand.
4. Add 8 mL of 2% FBS medium (pre-warmed to 37°C) to the plate.
5. Gently rotate the plate to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
6. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.
7. Discard the supernatant.
8. Apply 2 mL of 2% FBS media (pre-warmed to 37°C) to the conical tube and resuspend the cells thoroughly.

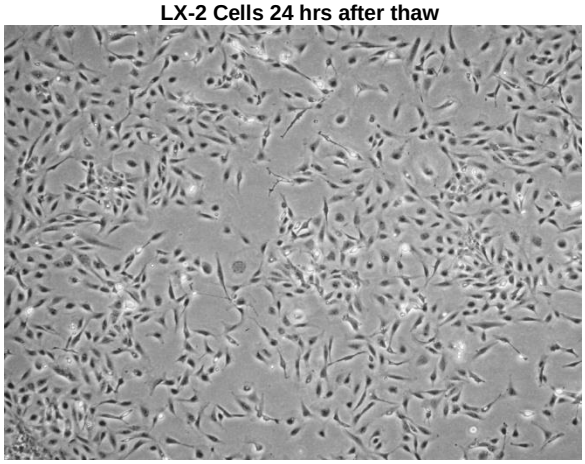
   IMPORTANT: Do not vortex the cells.
9. Count the number of cells using a hemocytometer.
10. Plate the cells to the desired density (typical split ratio is 1:3 to 1:6).

Cryopreservation of Cells

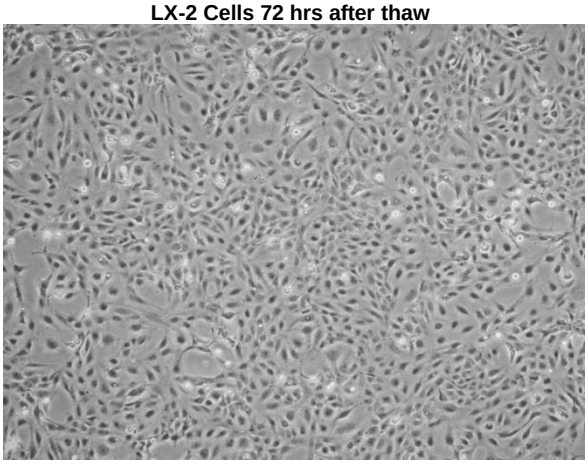
LX-2 cells grown in 2% FBS media can be frozen in 20% FBS growth media plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

Representative Lot Data

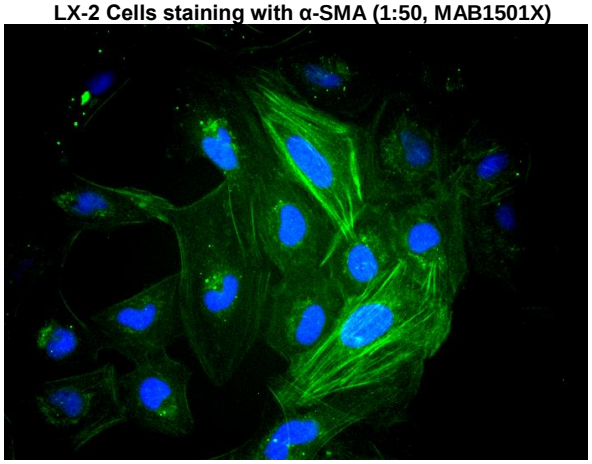
LX-2 Cells 24 hrs after thaw



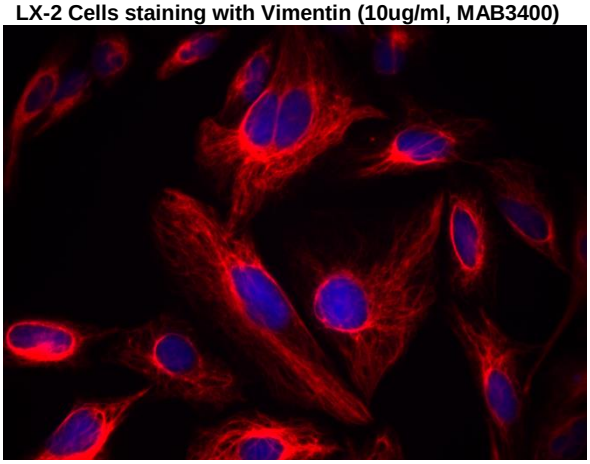
LX-2 Cells 72 hrs after thaw



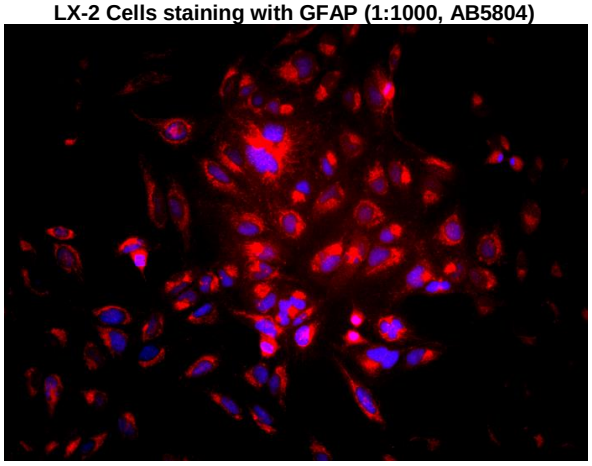
LX-2 Cells staining with α-SMA (1:50, MAB1501X)



LX-2 Cells staining with Vimentin (10ug/ml, MAB3400)



LX-2 Cells staining with GFAP (1:1000, AB5804)



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