Data Sheet

Assay Ready LX-2 Human Hepatic Stellate Cell Line

SCCAR10064-1VL

Pack Size: 1 vial

Store at Liquid Nitrogen

FOR RESEARCH USE ONLY

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

Assay ready cells cannot be expanded or passaged. Please see terms of use agreement below.

Background

Assay ready LX-2 cells are presented in a format that enables them to be thawed and grown directly in a 96-well plate for cell viability or other assays without the requirement of prior expansion in culture flasks or dishes.

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 immortalization 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*.

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: 8, 13, 15
FGA: 21, 23, 26	D5S818: 11, 12	TPOX: 8, 9	Penta E: 5, 21

Quality Control Testing

- Each vial contains ≥ 2.9 x 10^6 viable cells at thaw.
- The Assay Ready LX-2 cells are verified to be of human origin and negative for mouse, rat, Chinese hamster, Golden Syrian hamster, and non-human primate interspecies contamination, as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells tested negative for infectious disease against a Human Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

Storage and Handling

The LX-2 cells should be stored in liquid nitrogen. Upon recovery, the cells can be plated directly into one 96-well plate at a density of 30,000 live cells/well in their expansion medium. Treatments and viability assays can be performed 48 hours post plating the cells.

Do not expand, passage, or cryopreserve these cells after recovery.

Representative Data

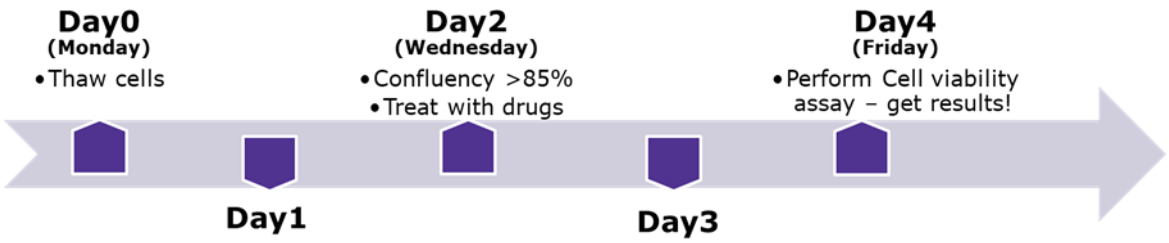


Figure 1. Approximate timeline for performing cell assay.

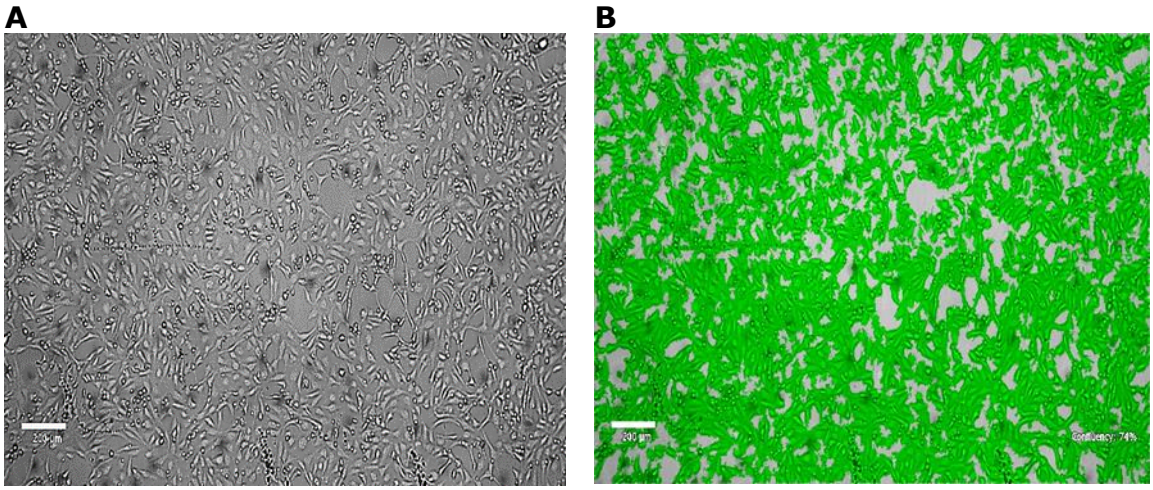
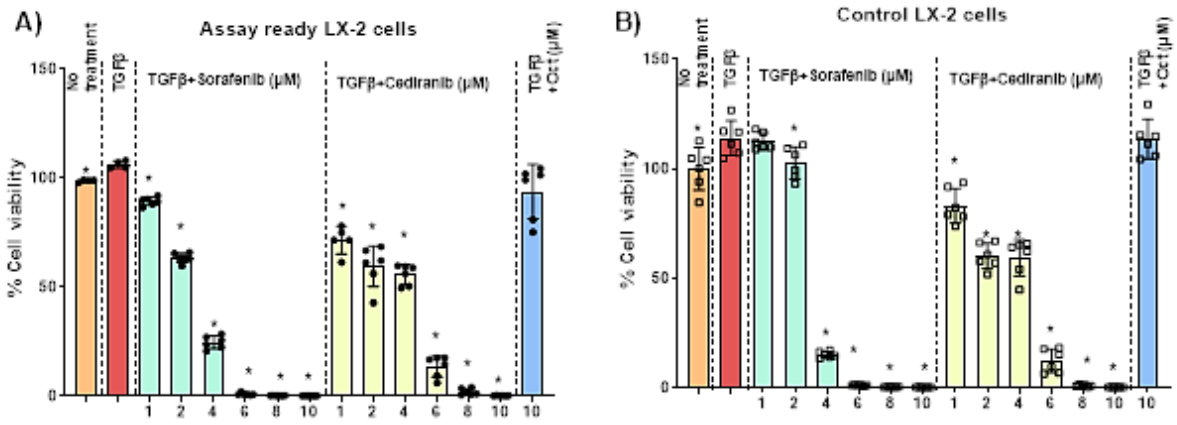


Figure 2. After thaw, LX-2 assay ready cells are plated at a density of 30,000 viable cells/well in a 96-well plate. (**A, B**) 24 hours post plating, cells are approximately 74% confluent as assessed by the Millicell® Digital Cell Imager (MDCI10000, 10X magnification). Cells are approximately 93% confluent by Day 2 post plating and can be treated with drugs (image not provided).



C)

	IC 50 (uM)	
	Sorafenib	Cediranib
Control LX-2 cells	2.936	6.540
ASR LX-2 cells	2.721	5.061

Figure 3. (**A**) Assay Ready LX-2 cells were plated in a 96-well plate at a density of 30,000 viable cells per well in medium containing 2% FBS and allowed to grow for 48 hours at 37 °C with 5% CO_2. The cells were then treated with or without TGFβ (40 ng/mL) and the drugs (Sorafenib, Cediranib or Octreotide at given concentrations) in medium containing 0.5% FBS and further incubated at 37 °C with 5% CO_2 for 48 hours. Cell viability was measured using CellTiter-Glo® Luminescent Cell Viability Assay (Promega G7571) as per kit manual. The values are normalized to no treatment group and represented as mean ± SD of percentage cell viability. (**B**) Control LX-2 cells that were in continuous culture were trypsinized and plated in a 96-well plate at 30,000 cells per well in medium containing 2% FBS. After 2-4 hours cells were treated with the designated drugs and cell viability measured following the same protocol as above. Statistical analysis was performed in GraphPad Prism using ANOVA. '*' Represents significant difference ($p \leq 0.05$) in cell viability as compared to TGFβ treated group. (**C**) IC_{50} of drugs were calculated in GraphPad Prism using Dose-response-variable slope (four parameters) model.

Protocols

Thawing of Cells

1. Do not thaw the cells until the recommended medium is on hand.

   Cells are thawed in DMEM High Glucose (D5671) medium containing 2% FBS (ES009-B), 1X Pen/Strep (P4333) and 1X Glutamine (TMS-002-C).
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 9 mL of 2% FBS medium (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. Be careful not to 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 small volume of 2% FBS medium (Step 1 above, pre-warmed to 37 °C).
10. Count cells using hemacytometer. Plate onto a 96-well plate at a density of 30,000 viable cells per well in 100 µL volume of 2% FBS medium.
11. Incubate the cells at 37 °C in a 5% CO_2 humidified incubator.
12. Monitor cell confluency using the MilliCell® Digital Cell Imager (MDCI10000). One day after plating, cells should be approximately 75% confluent.
13. On day 2 (48 hours post plating), when the cells are approximately 90% confluent, they can be treated with compounds of interest and can subsequently be used for viability or other assays.

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.

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