Data Sheet

LX-2 Cas9 Human Hepatic Stellate Cell Line

Immortalized Cell Line

SCC613

Pack Size: ≥ 1 x 10^6

Store in Liquid Nitrogen

FOR RESEARCH USE ONLY

Not for use in Diagnostic Procedures. Not for Human or Animal Consumption.

Background

Hepatic stellate cells perform vital roles in response to injury in the liver and are the body's major storage site for vitamin A. However, activation of hepatic stellate cells into fibrogenic myofibroblast-like cells in response to infection such as hepatitis and chronic conditions such as chronic alcoholism, fatty liver disease, obesity and diabetes causes liver fibrosis. Advanced liver fibrosis results in cirrhosis and liver failure. Given the importance of stellate cells in disease etiology, genetically malleable cellular models are critical for understanding the contribution of these cells to liver pathologies and necessary for development of new therapeutics.

The immortalized LX-2 cell line was established by Xu et al. to provide a stable, homogenous, and unlimited source of human hepatic stellate cells and overcome issues with culture variability. Since its introduction, LX-2 has proven a successful and physiologically relevant model for both clinical and basic research and development, as LX-2 cells retain key features of cytokine signaling, neuronal gene expression, retinoid metabolism, and fibrogenesis. The Cas9 version of the LX-2 cell line has been developed to allow targeted CRISPR/Cas9 modification and knockouts of genes in LX-2 cells. LX-2 Cas9 human hepatic stellate cells express a Cas9 cassette and transfection with CRISPR-cassettes to genes of interest enables direct assessment of genes associated with fibrogenesis and stellate cell disease response. The LX-2 Cas9 hepatic stellate cell line expresses common stellate cell markers such as MMP2 and alpha-smooth muscle actin while exhibiting slightly slower growth than original LX-2 cells in low-serum media.

Products in this document can be purchased at SigmaAldrich.com using the catalogue numbers in parenthesis unless otherwise noted.

Source

LX-2 Cas9 human hepatic stellate cells were derived from LX-2 cells transformed with a Cas9 cassette.

Short Tandem Repeat (STR) Profile

D3S1358: 13, 15	D21S11: 28, 31	TH01: 9.3	Penta D: 13, 15
D7S820: 11	D18S51: 12	TPOX: 8 ,9	Penta E: 5, 21
vWA: 17	D5S818: 11, 12	CSF1P0: 10, 12	
FGA: 13	D13S317: 11, 13	AMEL: X, Y	
D8S1179: 13	D16S539: 13		

Quality Control Testing

- Each vial contains ≥ 1 x 10^6 viable cells.
- Cells are tested negative for infectious diseases by a Human Essential Cell Line Examination and Report (CLEAR) Panel by Charles River Animal Diagnostic Services.
- Cells are verified to be of human origin and negative for inter-species contamination from mouse, rat, Chinese hamster, Golden Syrian hamster, and Non-human Primate (NHP) as assessed by a Contamination CLEAR Panel by Charles River Animal Diagnostic Services.
- Cells are negative for mycoplasma contamination.

Storage and Handling

LX-2 Cas9 human hepatic stellate cells should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting functionality.

Representative Data

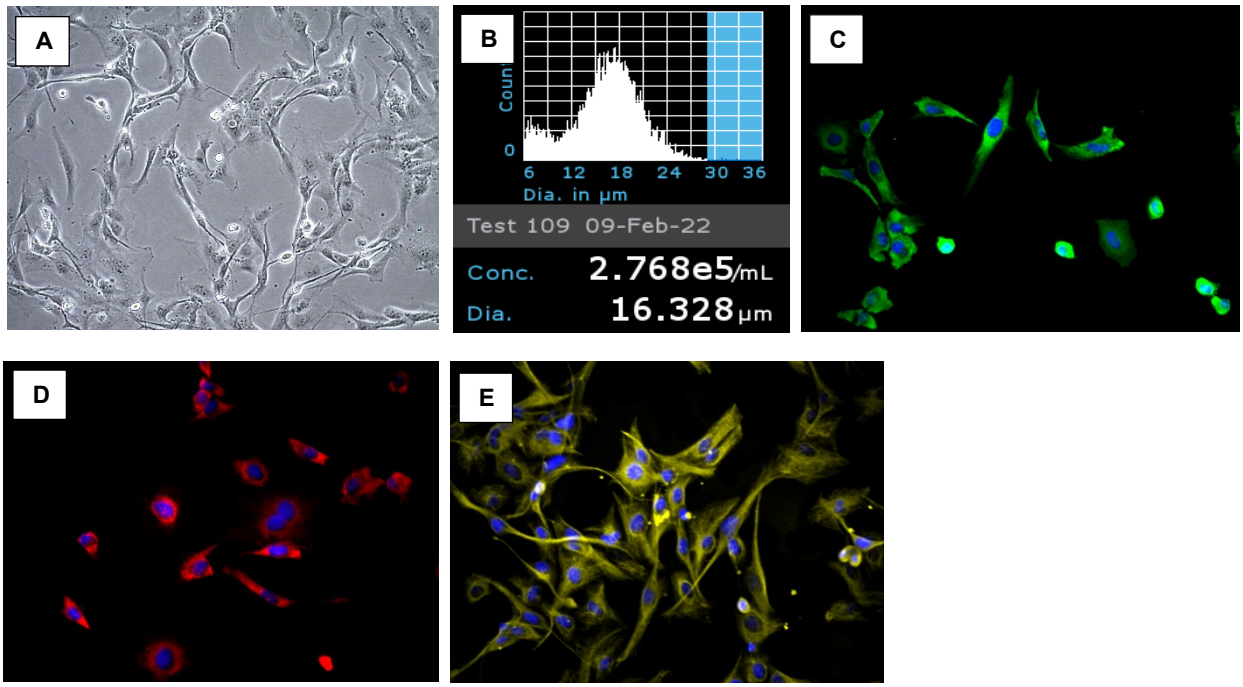
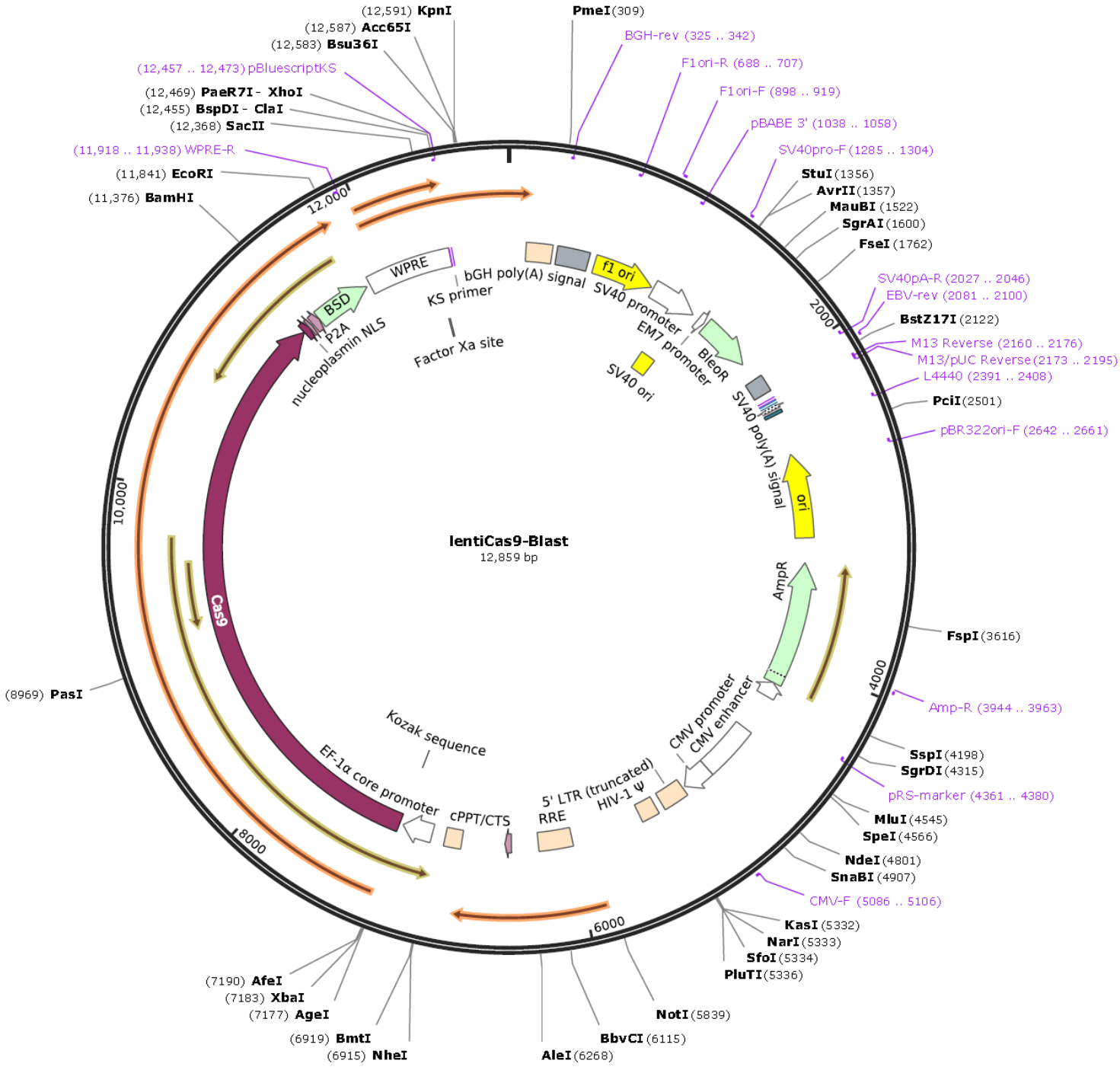


Figure 1: A. Bright-field image of LX-2 Cas9 cells one day after thaw in a T75 flask. B. Cell counting was performed using Scepter™ 3.0 Handheld Automated Cell Counter using 60 µm sensor tips (PHCC360KIT). C. LX-2 Cas9 cells express α-smooth muscle actin (SMA), D. matrix metallopeptidase 2 (MMP2) and E. Vimentin.

Figure 2: Schematic map of LentiCas9-Blast Vector



Protocol

Thawing Cells

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

   Cells are thawed and expanded in LX-2 Cas9 Expansion Medium comprised of DMEM-High Glucose medium (D5796) with 10% FBS (ES-009-B), 2 mM L-Glutamine (TMS-002-C), and 1X penicillin/streptomycin (TMS-AB2-C, optional).
2. Remove the vial of frozen LX-2 Cas9 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 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 mL or 2 mL pipette to transfer the cells to a sterile 50 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 LX-2 Cas9 Expansion Medium (Step 1 above) to the 15 mL conical tube.

   IMPORTANT: Do not add the entire volume of media all 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 up and down twice. Be careful not to introduce any bubbles.

   IMPORTANT: Do not vortex 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 15 mL of LX-2 Cas9 Expansion Medium.
10. Transfer the cell mixture to a T75 tissue culture flask.
11. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.

Subculturing Cells

1. LX-2 Cas9 cells should be passaged at ~80-85% confluency. Do not allow the cells to grow over 85% confluency.
2. Carefully remove the medium from the T75 flask containing the 80% confluent layer of LX-2 Cas9 cells. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
3. Apply 3-5 mL of Accutase® or Trypsin/EDTA solution and incubate in a 37 °C incubator for 3-5 minutes.
4. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
5. Add 8 mL of LX-2 Cas9 Expansion Medium to the plate.
6. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
7. Centrifuge the tube at 300 x *g* for 3-5 minutes to pellet the cells.
8. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
9. Apply 2-5 mL of LX-2 Cas9 Expansion Medium to the conical tube and resuspend the cells thoroughly.

   IMPORTANT: Do not vortex the cells.
10. Count the number of cells using a hemocytometer or a Scepter™ 3.0 Handheld Automated Cell Counter using 40 µm sensor tips.
11. Plate the cells to the desired density. Typical split ratio is 1:5 to 1:6. The medium should be replaced every other day.

Cryopreservation of Cells

LX-2 Cas9 human hepatic stellate cells may be frozen in LX-2 Cas9 Expansion Medium and 10% DMSO using a Nalgene™ slow freeze Mr. Frosty® container.

References

1. *Gut* 2005, 54(1): 142-151.
2. *PloS One* 2013, 8(10): e75692.

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