Sigma-Aldrich®

Data Sheet

Huh-7.5 Tet-On Human Hepatocellular Carcinoma Cell Line

Cancer Cell Line

Cat. # SCC265

pack size: ≥1x10^6 viable cells/vial

Store at: liquid nitrogen

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

Background

Hepatitis C virus (HCV) is an enveloped, positive sense, single-stranded hepatotropic RNA virus of the *Flaviviridae* family that is a major cause of liver disease such as fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) worldwide. There is no effective vaccine against HCV and approximately 80% of newly infected individuals, unable to clear the virus, develop chronic hepatitis.[1]

The establishment of Huh-7 cells and derivatives, the only cells permissive to HCV *in vitro*, has been instrumental in the elucidation of the HCV life cycle and enabling the screening and development of novel HCV-specific antivirals.

Source

Huh-7 is a well differentiated hepatocyte-derived carcinoma cell line, isolated from the liver tumor of a 57-year-old Japanese male in 1982.[2] Derived from Huh-7, the Huh-7.5 cell line is highly permissive for HCV RNA replication[3] and is now employed for high yield production of HCV and studies of antivirals on industrial scale.[4]

Huh-7.5 Tet-On cells were generated from Huh-7.5 using retroviral transduction to express the Tet-On 3G transactivator protein and enable subsequent doxycycline-regulated transgene expression to aid in the study of subcellular interactions between HCV and its host.[5,6]

Short Tandem Repeat (STR) Profile

D3S1358: 15	Penta E: 11	D16S539: 10	D8S1179: 14
TH01: 7	D5S818: 12	CSF1PO: 11	TPOX: 8, 11
D21S11: 30	D13S317: 10, 11	Penta D: 12	FGA: 22
D18S51: 15	D7S820: 10, 11	vWA: 16, 18	Amelogenin: X

Cancer and genetically modified cell lines are inherently genetically unstable. Instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passages.

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Quality Control Testing

- Each vial contains ≥ $1X10^6$ viable cells.
- Cells are tested negative for infectious diseases by a Human Essential 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

Huh-7.5 Tet-On 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

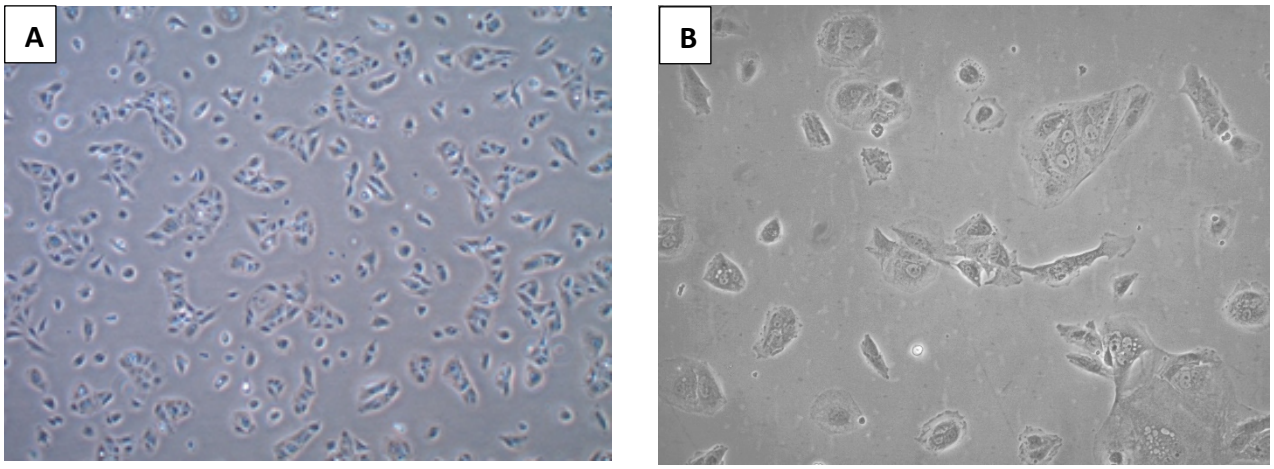

Figure 1. Bright-field images of cells one day after thaw. Lower (A) and higher (B) magnification.

Protocols

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.

   Huh-7.5 Tet-On Expansion Medium: Cells are thawed and expanded in Huh-7.5 Tet-On Expansion Medium comprising High Glucose DMEM (Sigma SLM-120-B, wih L-glutamine) supplemented with 10% Fetal Bovine Serum (Sigma ES-009-B), 1x NEAA (Sigma TMS-001), and 500 μg/mL of the selection antibiotic G418 / Geneticin (Sigma G8168).
2. Remove the vial of frozen Huh-7.5 Tet-On 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 Huh-7.5 Tet-On 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 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 15 mL of Huh-7.5 Tet-On 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. Huh-7.5 Tet-On cells should be passaged at ~70-80% confluency. Do not allow the cells to grow over 70-80% confluency. Throw out the cells if they become overgrown (greater than 80-90% confluent) or over-trypsinized.
2. Carefully remove the medium from the T75 tissue culture flask containing the 80% confluent layer of Huh-7.5 Tet-On cells.

3. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
3. Apply 5-7 mL of Accutase 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 5-7 mL of Huh-7.5 Tet-On 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 Huh-7.5 Tet-On 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.
11. Plate the cells to the desired density. Typical split ratio is 1:6.

Cryopreservation of Cells

Huh-7.5 Tet-On Human Hepatocellular Carcinoma Cell Line may be frozen in Huh-7.5 Tet-On Expansion Medium and 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

References

1. *Lancet* 2019; 394 (10207): 1451–66
2. *Cancer Res.* 1982; 42(9):3858-63.
3. *J. Virol.* 2002; 76(24):13001-13014
4. *Sci. Rep.* 2018; 8(1):17505.
5. *Cell.* 2015; 160(6): 1099-1110
6. *Cell Rep.* 2017; 21(2): 431–441.

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