

Human Hepatic Stellate Cells

From: Duke/UNC/UTA/EBI ENCODE group

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Hepatic stellate cells (HSCs) are the primary site for retinoid storage in the body and constitute approximately 8%–14% of cells in the normal liver. However, following injury, HSCs transdifferentiate into contractile and highly proliferative myofibroblast-like cells from their quiescent, lipocyte-like state. This process of transdifferentiation or activation is considered the crucial event that promotes increased extracellular matrix production and hepatic fibrosis as myofibroblastic HSCs are considered the primary source of collagen deposition in injured liver.

1. Source of Cells: Duke University Medical Center via CellzDirect (Invitrogen) from an untransplantable liver (Individual Hu8084). Requests for Hu8084 hepatic stellate cells (HSC) should be directed to Steve Choi (steve.choi@duke.edu).
2. Preparation of Cells: Liver is perfused with collagenase and dispersed cell suspensions were layered on a discontinuous density gradient of 5.8% Larcoll (Sigma-Aldrich) and 15.6% Histodenz (Sigma-Aldrich). The resulting upper layer consisted of >98% HSC (See Choi et al., 2009 for further details).
3. Donor Information: 59-year old woman
4. Karyotype: normal
5. Media for Cells: 1× Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, Catalog # 11960-044). Cells are grown in media supplemented with 10% Fetal Bovine Serum (Atlanta Biologicals Premium FBS, Catalog # S11150) and 1× Penicillin-Streptomycin (Invitrogen, Catalog # 15070-063).
6. Culture Conditions: Cells grow on standard culture plates and should be incubated at 37°C in the presence of 5% CO₂.
7. Cell Line Maintenance: Cells for passage are transferred to 50 mL conical tubes and centrifuged at 500 ×g for 5 minutes in a standard benchtop centrifuge with swing-out rotor. Media is aspirated and the stellate cells are resuspended at a density of 3 × 10² cells/mm².

Cells may be frozen at a density of 10⁶ cells/mL in 1× DMEM supplemented with 50% FBS and 10% DMSO. To freeze cells, pellet cells as if passaging, then resuspend in freezing medium and freeze at a rate of 1°C/min, then store in the vapor phase of liquid nitrogen. Frozen cells are recovered by thawing in a 37°C water bath, washing once with PBS or 1× DMEM, and resuspending in culture media.
8. Cell Passage Number: Cells are harvested at passage 8 or 9. These primary cells have been grown out to passage 20 without any noticeable signs of senescence.

References:

Choi SS, Omenetti A, Witek RP, Moylan CA, Syn WK, Jung Y, Yang L, Sudan DL, Sicklick JK, Michelotti GA, Rojkind M, Diehl AM. Hedgehog pathway activation and epithelial-to-mesenchymal transitions during myofibroblastic transformation of rat hepatic cells in culture and cirrhosis. *American Journal of Physiology Gastrointestinal Liver Physiology* 2009; 297(6): G1093-1106. PMID: 19815628