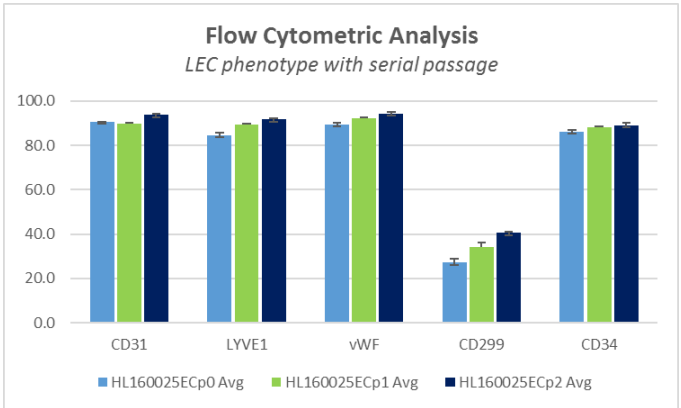
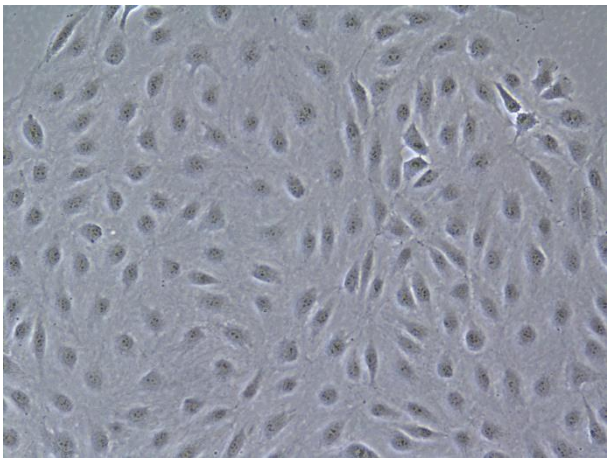
HUMAN LIVER-DERIVED ENDOTHELIAL CELLS (hLEC)

Samsara Sciences offers primary cryopreserved adult hLEC to support a broad array of research applications. hLEC can be propagated using industry-standard protocols for endothelial cells, and retain an endothelial cell morphology through limited serial passage. Phenotypic markers associated with liver-derived endothelial cells are expressed by hLEC and can be assessed using standard flow cytometric analyses or immunocytochemical analyses. Isolated populations of hLEC generally comprise cells that are immunopositive for CD31, CD32b, vWF, LYVE-1, CD34, and CD299 (L-SIGN), though the specific expression patterns and proportions of cells positive for a particular marker may vary from lot to lot due to inherent differences in donor characteristics. Phenotypic shifts are also common with extended time in culture and/or serial passage, and can depend greatly on culture conditions.

Phenotypic Stability

Flow cytometric analysis[1] reveals retention of key markers over serial passage *in vitro*. Endothelial cell markers, CD31 and von Willebrand factor (vWF), are expressed at a high level (generally >80% of the population) and not diminished with subculture. hLEC also express LYVE1 and a subpopulation (usually <50%) express CD299 (L-SIGN) – both markers associated with sinusoidal endothelial cells. Expression of CD45 and CD146 is minimal in cultured hLEC, typically <10% of the total population. Isolated hLEC consistently express high levels of CD34 as well. Consistency in phenotype among preparations from different donors can be appreciated in the hLEC Inventory Table (reverse).

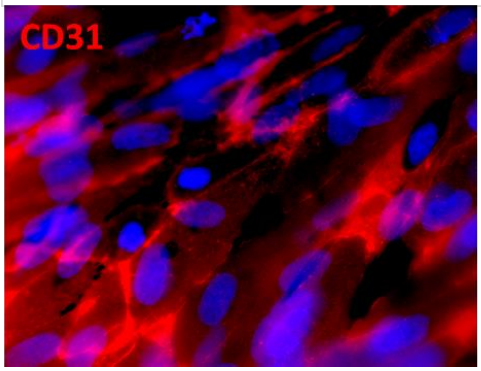


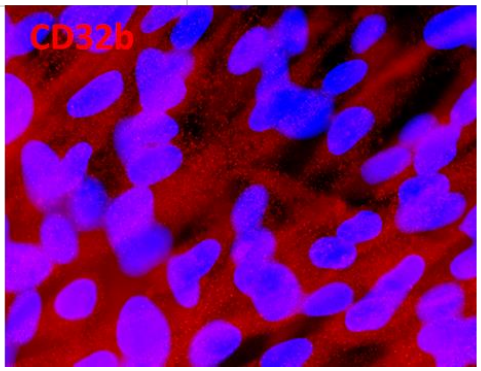


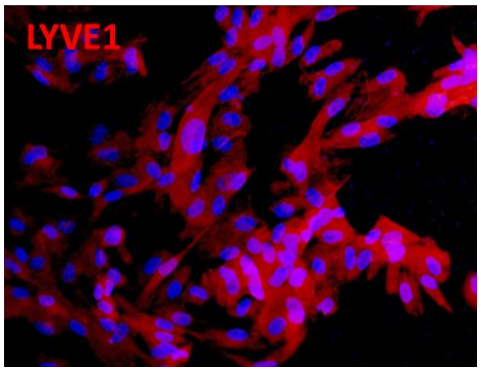
Phase contrast image (200x), lot HL160026EC[1]

Immunofluorescent detection of CD32b, LYVE1, & CD31

Immunocytochemical analysis[3] of cultured hLEC confirm expression of CD31 and LYVE1, as detected by flow cytometry. CD32b was also detected in isolated hLEC, suggestive of a sinusoidal phenotype.







LOT ID	LIVER PATHOLOGY	DONOR AGE / GENDER	DONOR MORBIDITIES	PHENOTYPE (BY FLOW CYTOMETRY) %Positive ± SEM															
				CD31			LYVE1			vWF			CD299			CD34		
				p0	p1	p2	p0	p1	p2	p0	p1	p2	p0	p1	p2	p0	p1	p2
HL160016EC	Near normal	0.66 / M	Muscular Dystrophy	nd	88.4 ± 0.6	96.8 ± 0.1	nd	89.6 ± 0.4	95.6 ± 0.3	nd	88.0 ± 0.3	93.5 ± 0.2	nd	60.0 ± 3.7	70.0 ± 1.2	nd	88.6 ± 0.8	93.7 ± 0.2
HL160017EC	Steatosis, cholangitis	31 / M	Chronic Alcoholism, Hypertension	nd	92.5 ± 0.8	91.4 ± 1.5	nd	96.0 ± 0.01	89.4 ± 1.0	nd	95.5 ± 0.4	93.1 ± 0.2	nd	71.6 ± 0.6	57.0 ± 1.7	nd	95.2 ± 0.2	91.4 ± 0.7
HL160024EC	Inflammation (chronic), Sepsis (possible), biliary obstruction	62 / M	Diabetes (Type 2), Hypertension, Gout	91.6 ± 1.23	88.6 ± 1.35	96.0 ± 0.4	79.2 ± 2.91	86.2 ± 1.2	85.2 ± 6.2	92.2 ± 0.4	90.4 ± 0.6	96.4 ± 0.4	54.6 ± 0.9	44.1 ± 0.5	44.9 ± 5.7	84.6 ± 0.9	84.8 ± 0.3	92.8 ± 0.5
HL160025EC	Inflammation (mild), glycogen accumulation	70 / M	None	90.7 ± 0.02	89.7 ± 0.6	93.6 ± 0.4	84.7 ± 0.9	89.3 ± 0.5	91.8 ± 0.3	89.4 ± 0.8	92.0 ± 0.5	94.3 ± 0.6	27.2 ± 1.6	34.3 ± 1.9	40.4 ± 0.7	86.2 ± 0.8	88.2 ± 0.3	89.1 ± 1.0
HL160026EC	Near normal	25 / F	Pulmonary Arterial Hypertension Lung Transplant	76.7 ± 1.8	91.7 ± 0.5	94.0 ± 0.7	89.4 ± 1.2	88.6 ± 1.7	91.0 ± 0.6	92.6 ± 0.3	88.1 ± 0.3	92.0 ± 0.9	64.0 ± 0.7	63.4 ± 2.0	57.7 ± 0.4	90.5 ± 0.5	85.0 ± 0.9	88.6 ± 1.1

MATERIALS & METHODS

[1]Samples were fixed in 2% PFA in PBS and stained with antibodies to:

CD31 (Miltenyi 130-096-653)
CD45 (BioLegend 368512)
LYVE-1 (Novus Biologicals FAB20892V)
CD34 (Miltenyi 130-098-142)
CD146 (Miltenyi 130-099-956)
vWF (Abcam ab195028)
CD299 (BioLegend 845002)

Isotype controls were run for each marker, excepting LYVE-1 in which unstained cells served as negative controls. A MACSQuant Analyzer with MACSQuantify software (Miltenyi Biotec) was used for analysis.

[2]Recommended culture seeding density is 3,000 viable cells/cm^2, grown in EBM-2 with EGM-2 supplement (Lonza) on Collagen 1 BioCoat surface (Corning). Recommended confluence at the time of harvest/passage is 80-90%. Recommended dissociation reagents, 0.25% Trypsin or TrypLE™ (Thermo Fisher). Recommended centrifugation 300x g for 5 minutes. Lower seeding densities, alterations in media formulation, under- or over-confluence at the time of harvest, or continuous serial passage may alter cell morphology and phenotype.

[3]Cells were grown to confluence on Transwell plates (Corning) and fixed with 4% freshly-prepared paraformaldehyde for 20 minutes, washed in PBS and subjected to immunofluorescent labeling using 10µg/mL R&D Systems antibodies to CD31 (Clone 9G11), CD32b (AF1330), and LYVE1 (AF2089). Mouse IgG or Goat IgG were used as controls for CD31 and CD32 & LYVE-1, respectively. All cells were counterstained with DAPI and imaged.

[4]All histopathology assessment of liver tissues conducted by board certified liver pathologist and scored according to standard clinical practice.

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www.samsarasciences.com
info@samsarasciences.com

858-617-0790