INDEX FOR 240L PRODUCT SPECIFICATION SHEET

which are pooled to determine the fold increase over a given period of time. To determine average % CFUs, the number of colonies counted divided by the number of cells plated x 100 is calculated for three plates and averaged. The cells are differentiated for fat and bone at each Passage and for cartilage on Passage 2 cells and the results are indicated as: "+" (positive - clear presence of fat or bone (mineral) or cartilage), "±" (positive/negative – abnormal or sparse presence of fat or bone (mineral) or cartilage) and "-" (negative - total absence of fat or bone (mineral) or cartilage).

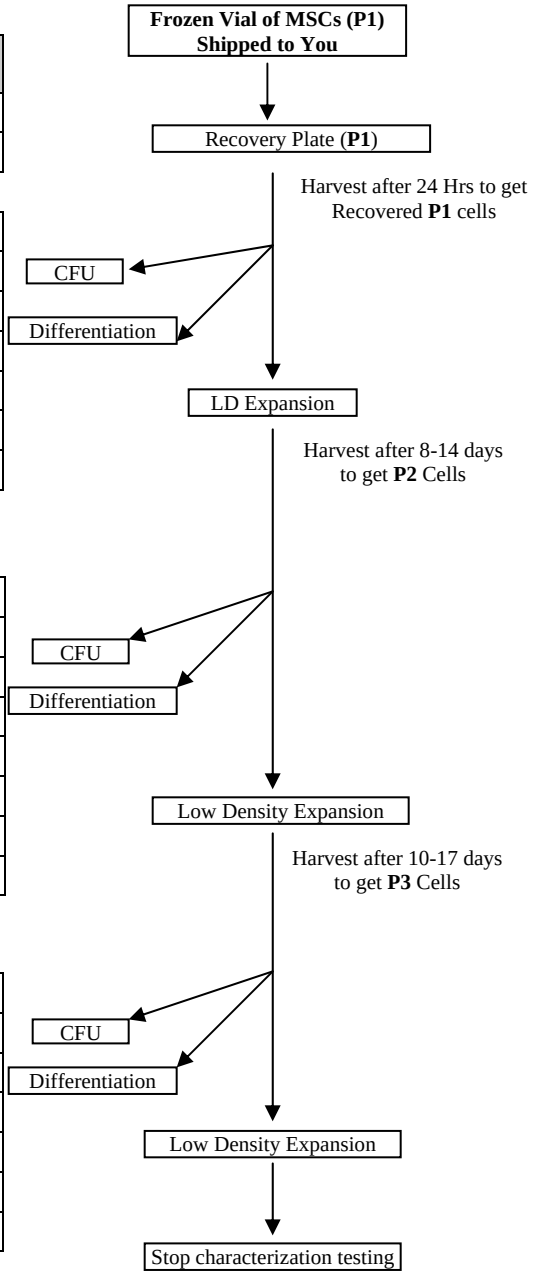
Results of Tests Done on Frozen hMSCs:

		Trial 1	Trial 2	Trial 3	Trial 4
Recovery Plate Info:					
Recovery of frozen P1 cells	**%:**	50	35	47	36.75

Assays done on Recovered P1 cells:					
CFUs	**%:**	76.7	50.3	68	76
Bone Differentiation	**Result:**	+	+	+	+
Fat Differentiation	**Result:**	+	+	+	+
LD Expansion	**Days:**	10	9	10	10
	Fold:	379	117	255	444
	Doublings:	8.55	6.9	7.99	8.8

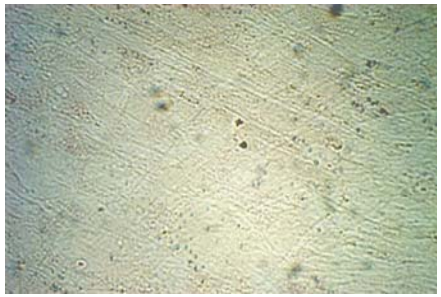
Assays done on Expanded P1 cells (P2 cells):					
CFUs	**%:**	30.6	37	41.7	13.3
Bone Differentiation	**Result:**	Thrown out inadvertently			
Fat Differentiation	**Result:**	Thrown out inadvertently			
Chondro Differentiation	**Result:**	N/A	N/A	N/A	+
LD Expansion	**Days:**	12	11	11	11
	Fold:	256	315.5	152	208
	Doublings:	8	8.3	7.2	7.7

Assays done on Expanded P2 cells (P3 cells):					
CFUs	**%:**	36.3	22.3	36.8	38.3
Bone Differentiation	**Result:**	N/A	N/A	+	+
Fat Differentiation	**Result:**	N/A	N/A	+	+
LD Expansion	**Days:**	13	14	10	10
	Fold:	177.6	237.9	259	261
	Doublings:	7.47	7.9	8.0	8.0



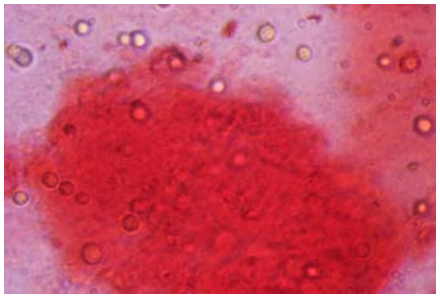
Note: This product is for research use only, and is not intended for therapeutic or diagnostic applications.

240L FAT and BONE Differentiation of Recovered Passage 1 Cells



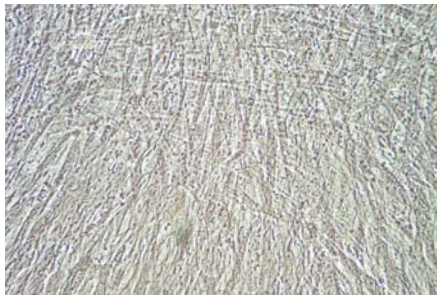
CONTROL, 20X

FAT, 40X

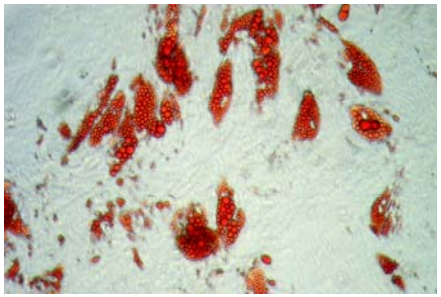


BONE, 40X

240L FAT and BONE Differentiation of Passage 3 Cells



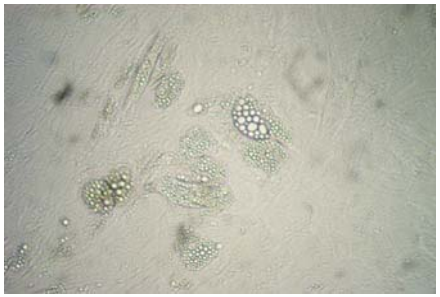
CONTROL, 10X



FAT, 10X

BONE, 10X

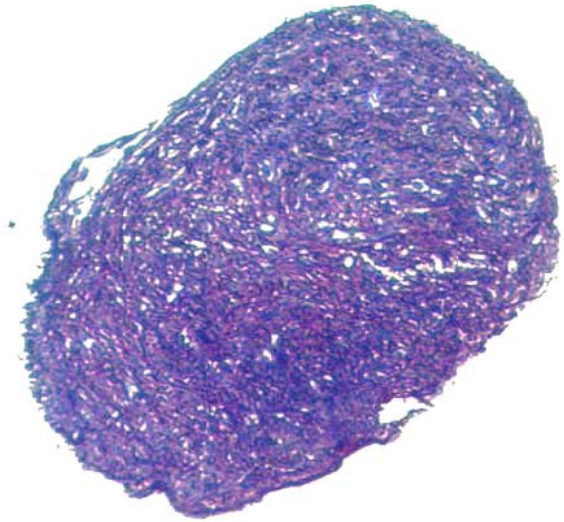
240L FAT and BONE Unstained Differentiation of Passage 3 Cells



FAT, 10X

BONE, 10X

240L Chondrogenic Differentiation of Passage 2 Cells



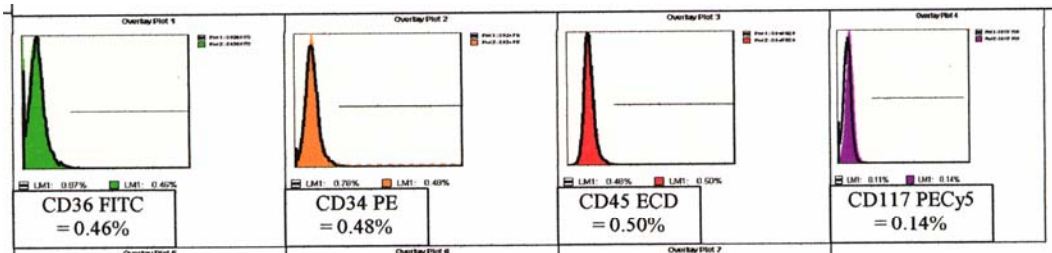
(4X, Toluidine Blue Sodium Borate stain)

FLOW CYTOMETRY MARKER RESULTS

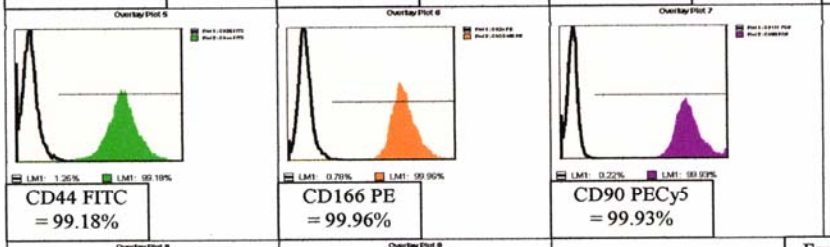
Panel No	Antibody	Expected Result on MSCs	Whole Bone Marrow (% Gated)	240L P2 (% Gated)
1	CD34 PE	Neg	1.2	0.48
	CD36 FITC	Neg	19.5	0.46
	CD45 ECD	Neg	95.8	0.50
	CD117 PC5	Neg	2.6	0.14
2	CD44 FITC	>Important Pos	93.3	99.18
	CD90 PC5	>Important Pos	2.1	99.93
	CD166 PE	>Important Pos	0.7	99.96
3	CD49b FITC	>Important Pos	3.6	5.85
	CD105 PE	>Important Pos	0.8	99.76
4	CD29 PC5	<Important Pos	43.1	99.39
	CD49c PE	<Important Pos	0.2	99.79
	CD147 FITC	<Important Pos	46.9	99.78
5	CD59 FITC	<Important Pos	45.9	99.81
	CD184 PE	Dim on MSCs	20.4	22.82
6	CD49f PC5	Dim on MSCs	0.2	56.87
	CD 106 PE	Dim on MSCs	3.1	18.60
	HLA-1:ABC FITC	Dim on MSCs	94.6	99.67

HISTOGRAMS OF FLOW MARKER PANELS WITH ISOTYPE CONTROLS

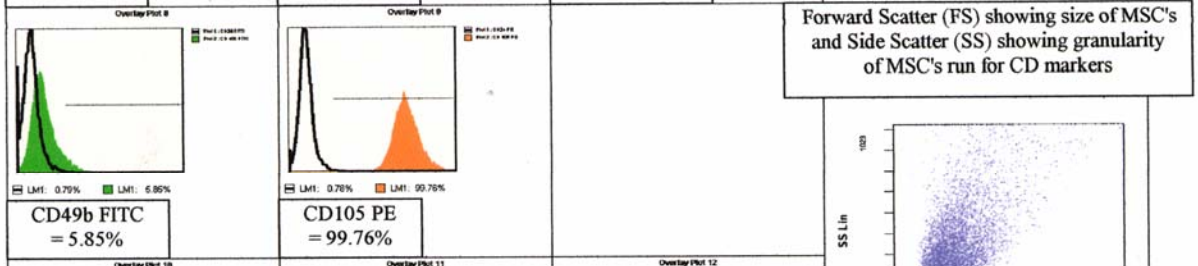
Panel 1



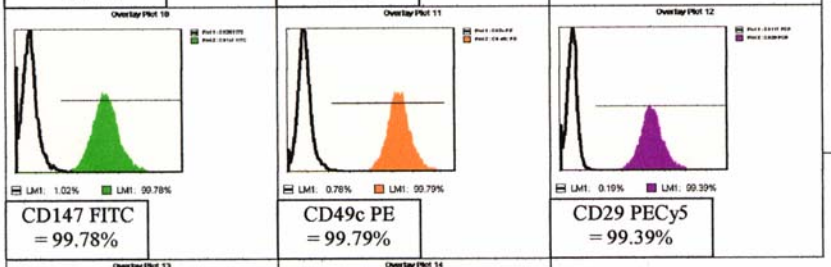
Panel 2



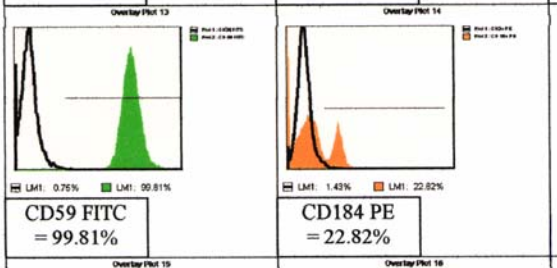
Panel 3



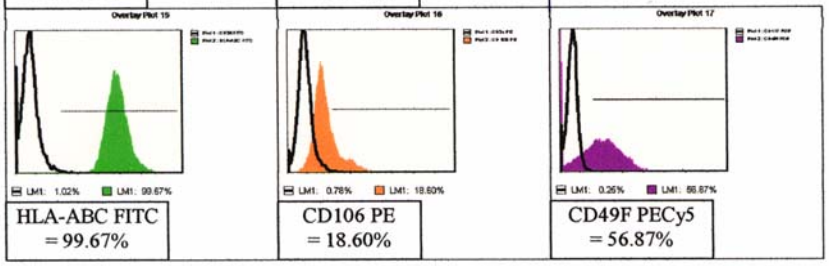
Panel 4



Panel 5



Panel 6



Specific Antibodies – Solid Colored Peaks
Isotype Controls – Black and White Peaks

TABLE OF MARKERS FOR HUMAN MSC
FLOW CYTOMETRIC ANALYSIS

MARKER	DESCRIPTION	POSITIVE ON	LABEL	VENDOR	CAT #/Isotype
CD29	fibronectin receptor	fibroblasts, platelets, T cells	**PC5**	BD	559882/ IgG1
CD34	Cell-cell adhesion molecule and cell surface glycoprotein	hematopoietic progenitor cells, vascular endothelial cells	**PE**	Coulter	IM1871/ IgG1
CD36	Aka platelet GPIV or GPIIIb; thrombospondin receptor. Cell adhesion molecule in platelet adhesion and aggregation, platelet-monocyte and platelet-tumor cell interaction	platelets, macrophages, endothelial cells, early erythroid cells, megakaryocytes	**FITC**	Coulter	IM0766/ IgG1
CD44	Family of cell surface glycoproteins with isoforms generated by alternate splicing of mRNA. Important in epithelial cell adhesion to hyaluronate in basement membranes and maintaining polar orientation of cells; also binds laminin, collagen and fibronectin	white blood cells, red blood cells, breast, colon, stomach, uterus, most tissue	**FITC**	Coulter	IM1219/ IgG1
CD45	leukocyte common antigen (LCA)	hematopoietic cells, stronger in lymphocytes	**ECD**	Coulter	IM2710/ IgG1
CD49b	very late antigen (VLA) alpha 2 chain - on T cells, Aka GPIa/IIa when expressed on platelets	platelets, activated B & T cells	**FITC**	Coulter	IM1425/ IgG1
CD49c	very late antigen (VLA) alpha 3 chain. Receptor for laminin, collagen, fibronectin, thrombospondin	nonhematopoietic cells	**PE**	BD	556025/ IgG1
CD49f	Aka very late antigen (VLA) alpha 6 chain; Laminin receptor	epithelial cells	**PC5**	BD	551129/ IgG1
CD59	Aka protectin Regulates complement mediated cell lysis by inhibiting formation of membrane attack complex (MAC)	most cells	**FITC**	Coulter	IM3457/ IgG1
CD90	Thy-1	hematopoietic stem cells, neurons, connective tissue	**PC5**	Coulter	IM3704/ IgG1
CD105	endoglin, Regulatory component of TGF-beta receptor complex; mediates cellular response to TGF-beta 1	activated monocytes, endothelial cells; erythroid precursors in marrow	**PE**	Coulter	A07414/ IgG1
CD106	Aka VCAM-1; alpha 4 beta 1 ligand	Adhesion molecular found in stimulated endothelium that plays a role in migration of white blood cells	**PE**	BD	555647/ IgG1
CD117	Aka c-kit, stem cell factor receptor	interstitial cells of Cajal, hemopoietic progenitor cells, melanocytes, embryonic/fetal brain, endothelium, gonads, mast cells, breast epithelium, germ cells	**PC5**	Coulter	IM2657/ IgG1
CD147	Aka neurothelin, extracellular matrix metalloproteinase inducer	all leukocytes, red blood cells, platelets and endothelial cells	**FITC**	BD	555962/ IgG1
CD166	Aka **A**ctivated **L**eukocyte **C**ell **A**dhesion **M**olecule (ALCAM)	neurons, activated T cells, activated monocytes, epithelium, fibroblasts	**PE**	Coulter	A22361/ IgG1
CD184	Aka CXCR4, **S**tromal cell **D**erived **F**actor 1 (SDF1). Receptor for the CXC chemokine SDF-1	all mature blood cells, blood progenitor cells, endothelial and epithelial cells, astrocytes, neurons	**PE**	Coulter	A07414/ IgG1
HLA-1, ABC	The antigen corresponds to a monomorphic determinant of human HLA class I molecules. HLA-ABC is associated with beta-2 microglobulin.	It is expressed on the surface of all human cell types.	**FITC**	Coulter	IM1838/ IgG2a
Isotype Controls	Mouse IgG1/Mouse IgG2a	Only when there is non-specific binding of the Mouse Ig	**PE/FITC**	Coulter	6604240