

Product Specification Sheet

Product: Human Marrow Stromal Cells (hMSCs) frozen at passage 1

Donor: 220R

Gender: Male

Ethnicity: Caucasian

Size: 1 ml

Number of vials: 2

Concentration: 1×10^6 cells/ml/vial

Freezing media: α MEM with 2 mM L-glutamine 65%, Fetal Bovine Serum 30%, DMSO 5%

Culture media: α MEM with 2 mM L-glutamine 83.5%, Fetal Bovine Serum 16.5%

If shipped on Dry Ice or Dry Ice Equivalent

Shipping: Dry ice for less than 72 hours.

Storage: Store vial(s) on dry ice for less than 72 hours or at -80°C for less than 1 week and/or plate immediately on recovery plates.

If shipped in Liquid Nitrogen Shippers

Shipping: Liquid nitrogen equivalent for less than 144 hours (6 days).

Storage: Transfer vial(s) to liquid nitrogen storage immediately upon receipt or plate on recovery plates immediately

Results from Infectious Agents Blood Screen:

Tests performed by LabCorp Burlington, NC

Test	Results
HIV-1 Antibodies	Negative
HIV-2 Antibodies	Negative
Hepatitis B Surface Antigen	Negative
Hepatitis B Core Antibodies	Negative
Hepatitis C Virus Antibodies	Negative
HTLV-I/II Antibodies	Negative
Cytomegalovirus Antibodies, IgM	Negative
Mycoplasma Antibodies	
IgG*	Positive*
IgM	Negative
Syphilis	Negative

*Elevated Mycoplasma IgG values are common. Positive values may indicate a recent infection with Mycoplasma pneumoniae and a current infection needs to be confirmed by a positive IgM result.

Sterility Testing of Frozen Samples: A frozen aliquot of this sample was cultured for sterility at the Tulane University Hospital and Clinic Pathology Lab. Cultures were performed for presence of aerobic bacteria, acid-fast bacteria (mycobacteria) and fungus. All cultures were negative, indicating no aerobic or acid fast bacteria or fungus could be detected in the frozen aliquot.

Analysis on Expansion of Frozen hMSCs: A bone marrow aspirate is drawn and mononuclear cells are separated using density centrifugation. The cells are plated to obtain adherent human marrow stromal cells, which are harvested when cells reach 70%-80% confluence. These are considered passage zero (P0) cells. These P0 cells are expanded, harvested and frozen at passage 1 (P1) for distribution. Prior to release, two trials of the frozen P1 cells are analyzed over three passages for Colony Forming Units (CFUs), cell growth, and differentiation into fat and bone. Two vials of frozen P1 cells are recovered for 24 hours on two 10 cm² plates which are pooled and expanded

for analysis. To determine cell growth, cells are plated at low density (LD) (50 cells/cm²) in two 10 cm² plates 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).

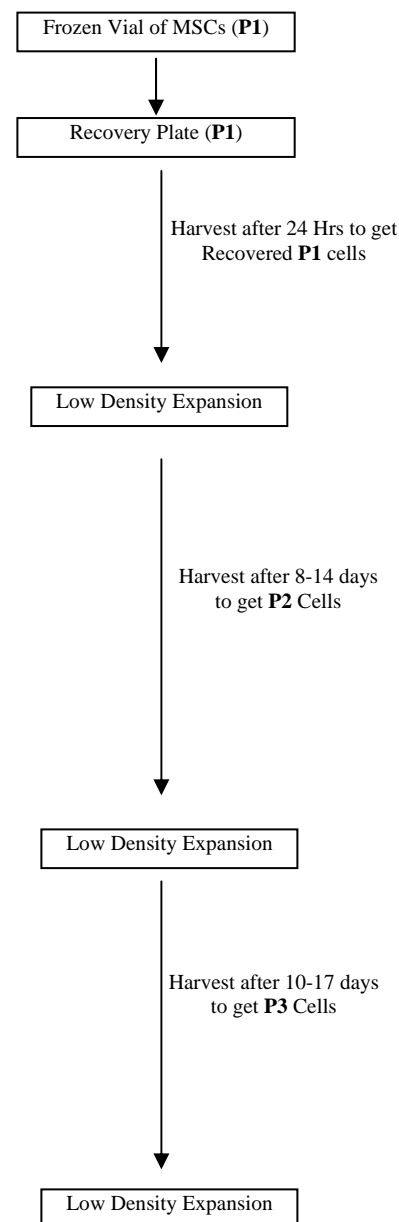
Results on Tests Done on Frozen hMSCs:

		Trial 1	Trial 2	Trial 3 *
Recovery of frozen P1 cells	%:	31.5	40.5	35

Assays done on Recovered P1 cells:				
CFUs	%:	43	45.3	68.3
Bone Differentiation	Result:	+	+	+
Fat Differentiation	Result:	+	+	+
LD Expansion	Days:	10	13	14
	Fold:	229	198	259
	Doublings:	7.84	7.63	8.0
	Passage on Harvest:	2	2	2

Assays done on Expanded P1 cells (P2 cells):				
CFUs	%:	55.3	35.7	19.7
Bone Differentiation	Result:	+	+	N/A
Fat Differentiation	Result:	+	+	N/A
Chondro Differentiation	Result:	NA	NA	+
LD Expansion	Days:	13	13	13
	Fold:	248	226	181
	Doublings:	7.95	8.26	7.5
	Passage on Harvest:	3	3	3

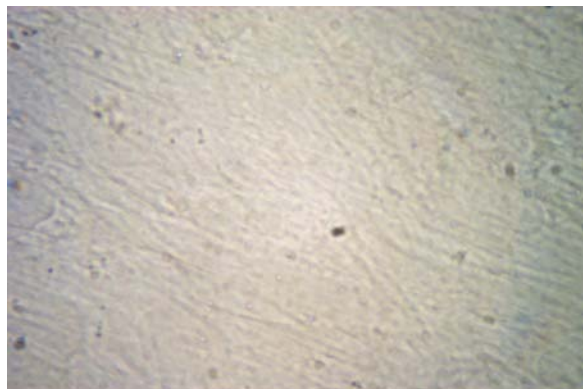
Assays done on Expanded P2 cells (P3 cells):				
CFUs	%:	25.3	31.7	28.3
Bone Differentiation	Result:	+	+	N/A
Fat Differentiation	Result:	+	+	N/A
LD Expansion	Days:	13	14	17
	Fold:	226	215.5	116
	Doublings:	7.81	7.75	6.9
	Passage on Harvest:	4	4	4



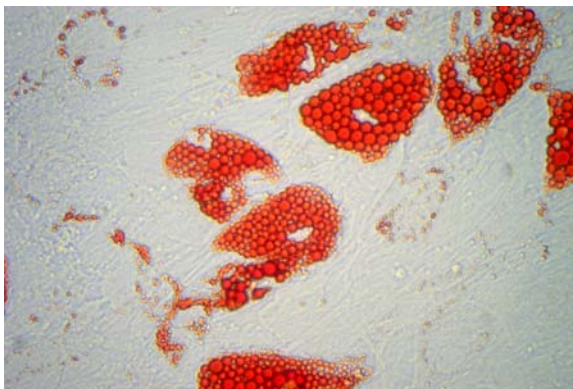
***Trial 3:** Data is from a frozen vial of P1 cells which was put onto dry ice for 48 hours (to simulate extended shipping time) and then thawed, recovered, expanded and differentiated.

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

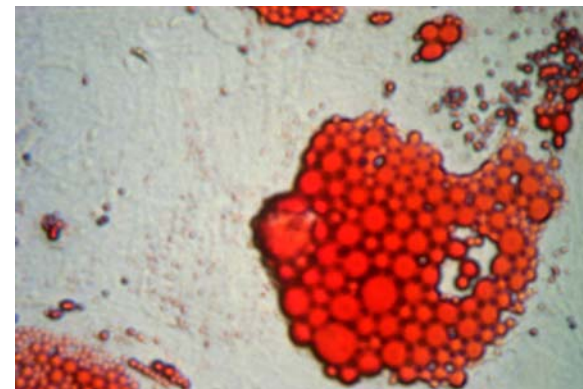
220R FAT and BONE Differentiation of Passage 1 Cells (Trial 1)



FAT Control 40X



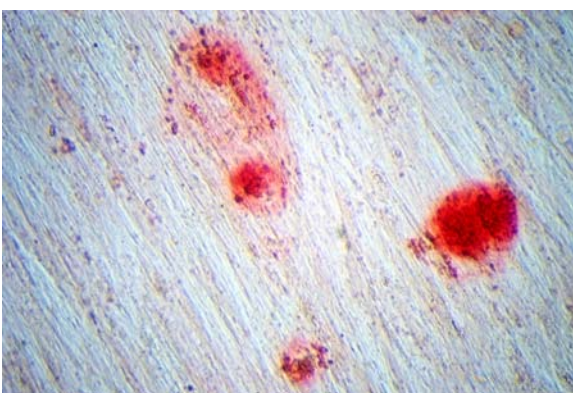
FAT 20X



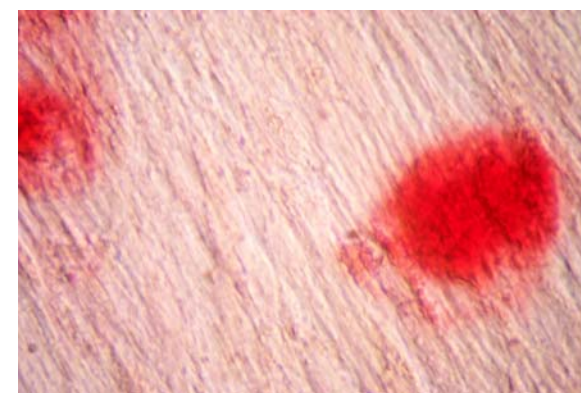
FAT 40X



BONE Control 40X

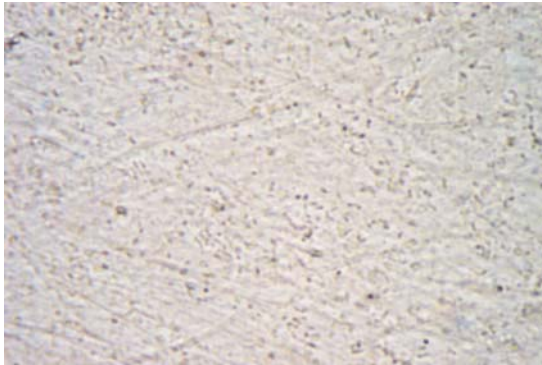


BONE 20X

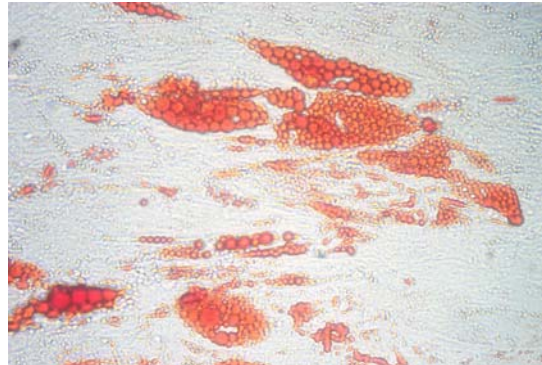


BONE 40X

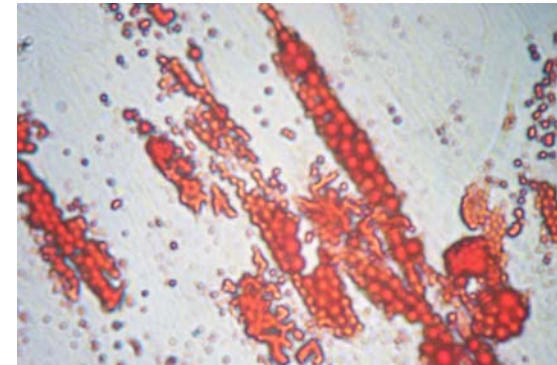
220R FAT and BONE Differentiation of Passage 2 Cells (Trial 1)



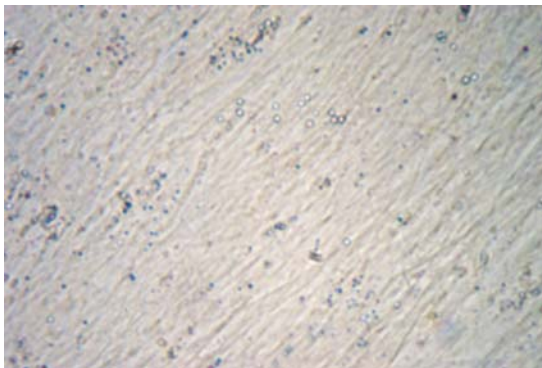
FAT Control 40X



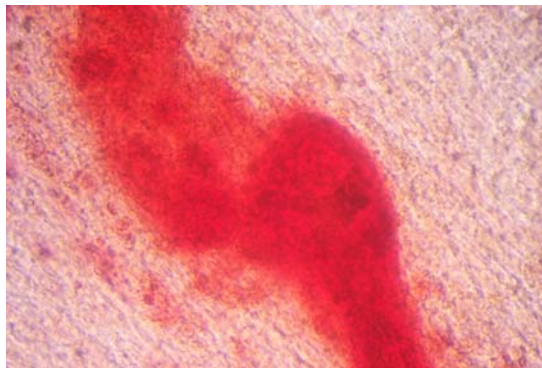
FAT 20X



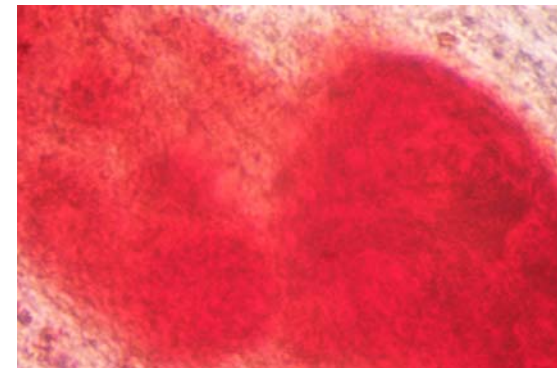
FAT 40X



BONE Control 40X

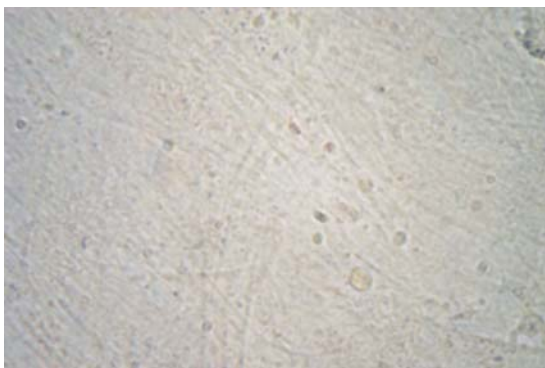


BONE 20X

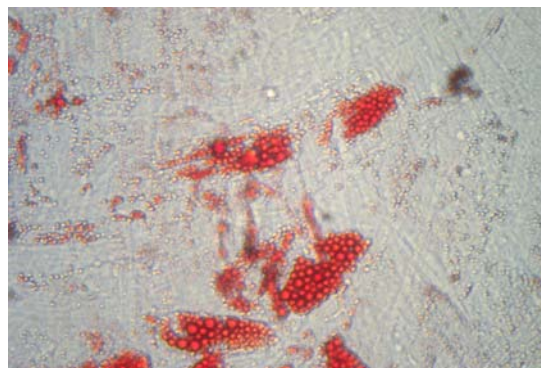


BONE 40X

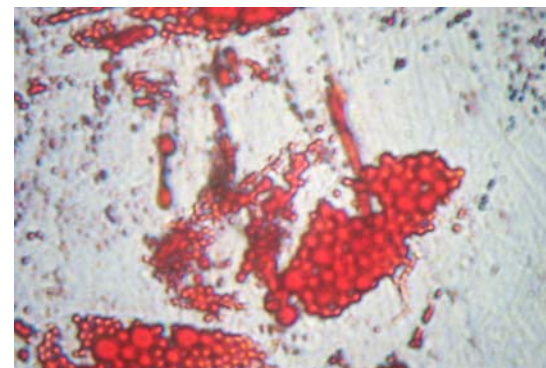
220R FAT and BONE Differentiation of Passage 3 Cells (Trial 1)



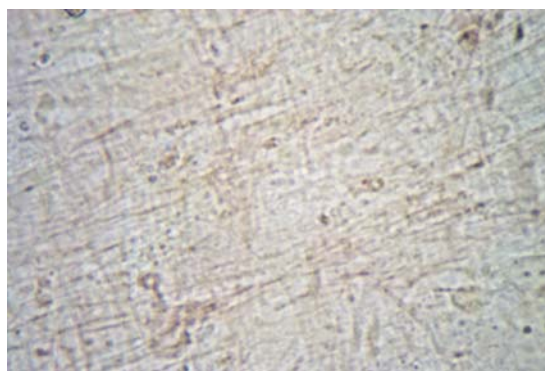
FAT Control 40X



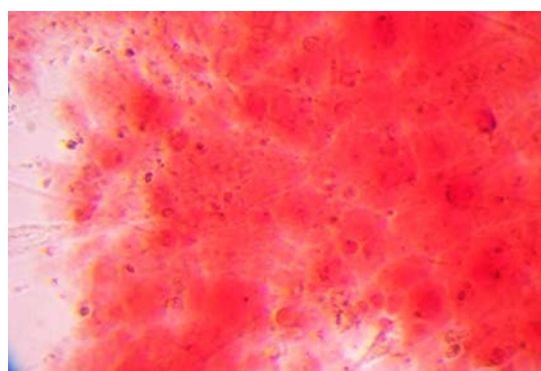
FAT 20X



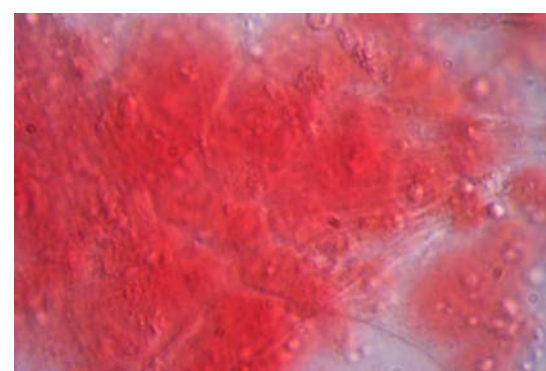
FAT 40X



BONE Control 40X

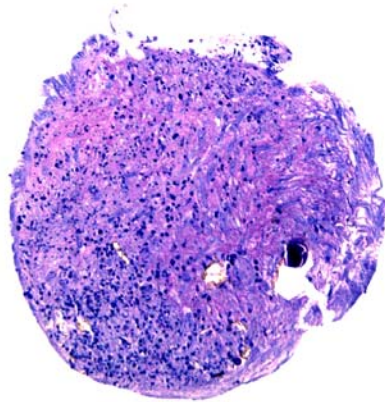


BONE 20X



BONE 40X

220R Chondrogenic Differentiation of Passage 2 Cells (Trial 3)



(10X, Toluidine Blue Sodium Borate stain)