

PeperoGrow™ hMSC Medium

Maintenance Media for
Human Mesenchymal Stem Cells



- ✓ Complete media
- ✓ Superior growth rates
- ✓ Xeno-free, phenol red-free
- ✓ Phenotypic integrity
- ✓ Maintained multipotency
- ✓ Affordable and competitive pricing
- ✓ Works well with hMSCs derived from:
 - Adipose tissue
 - Bone marrow
 - Umbilical cord
 - Urine
- ✓ Developed in collaboration with American CryoStem Corporation



**OUR SUPPORT
YOUR DISCOVERY**

PeproGrow™ hMSC Medium

PeproGrow™ hMSC (Mesenchymal Stem Cell) Medium is a xeno-free, human serum containing, phenol red-free complete media formulation originally designed for the in vitro expansion of adipose-derived human mesenchymal stem cells (ADMSCs) while maintaining full multipotency. Since its design, this media formulation has been shown to be suitable for the sustained growth of adipose tissue-derived, bone marrow-derived, umbilical cord-derived, placental-derived, and urine-derived MSCs in both adherent and suspension culture. For optimal results, culturing should be conducted on a surface coated with PeproTech's Animal-Free Human Vitronectin Matrix as a surface-coating reagent; however, other suitable extracellular matrix (ECM) proteins, such as fibronectin or vitronectin, can be used. PeproGrow hMSC Medium was designed and developed in collaboration with American CryoStem Corporation, and is supplied as a 500mL bottle of PeproGrow hMSC Basal Medium containing a human serum component, and a separate, lyophilized vial of animal-free PeproGrow hMSC Growth Factor Supplement. The addition of the separate, lyophilized growth factor supplement to the basal medium results in a complete medium containing all growth factors and supplements necessary for optimal expansion of human mesenchymal stem cells in culture. Additional companion products, including PeproTech's Animal-Free Vitronectin Matrix and Buffer Kit, are available separately.

Desired Attributes of hMSC Media:

- Sustain rapid, long-term expansions
- Maintain immunophenotype and stemness
- Maintain multipotent potential
- Maintain normal physiological functions, including secretion of cytokines, chemokines, and growth factors

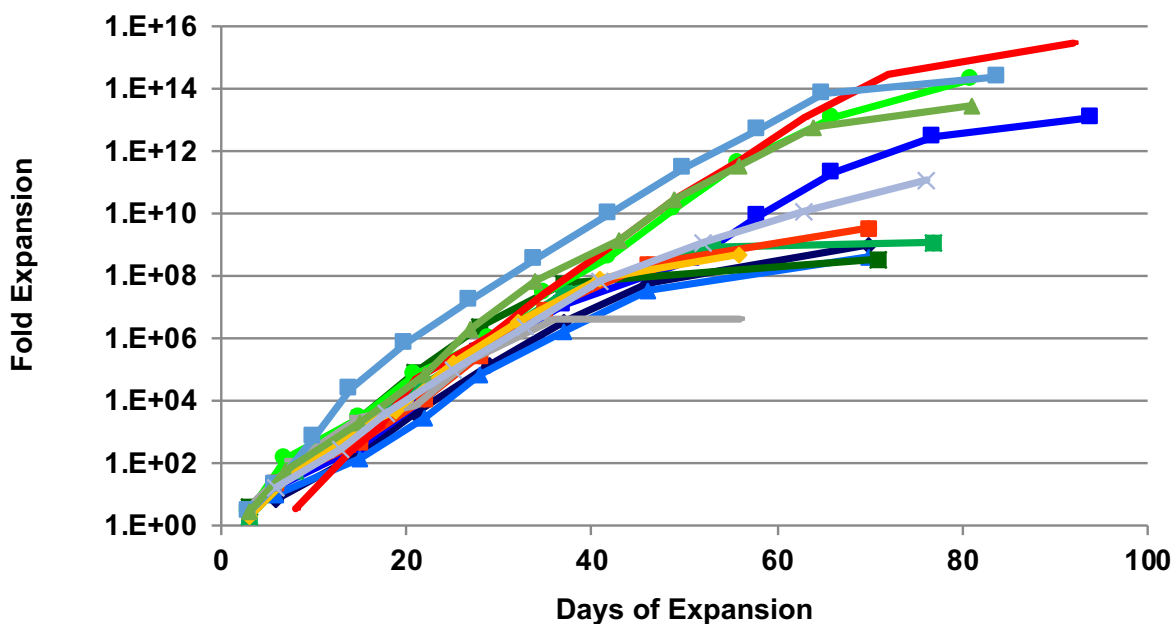
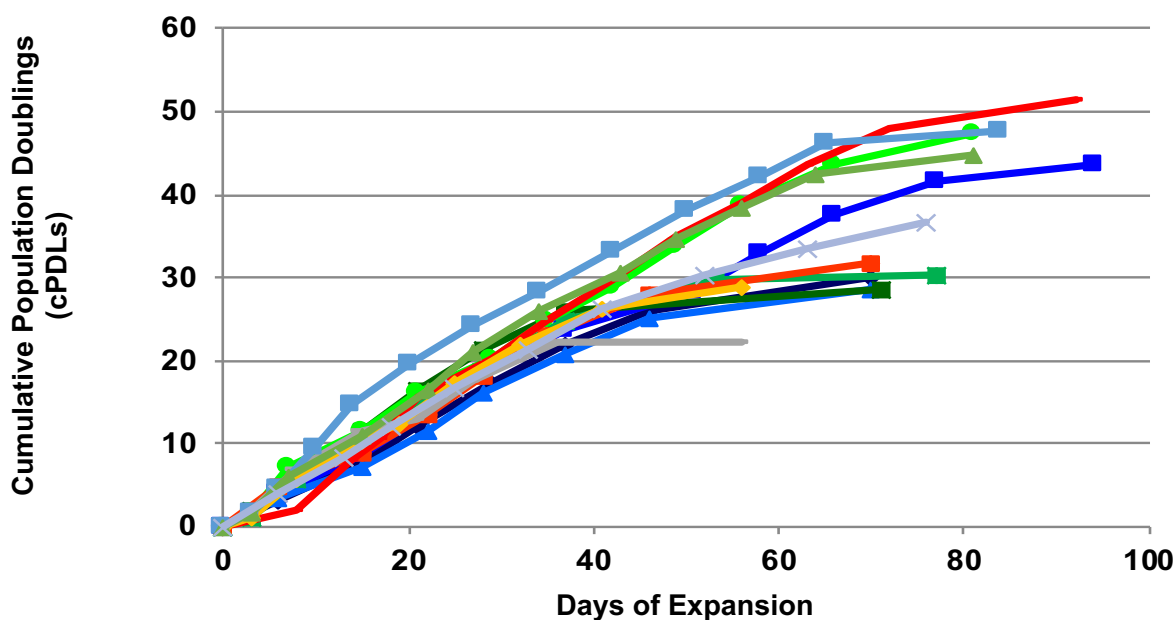
In the human body, hMSCs grow throughout life while maintaining these functional characteristics.... **realistic data demands realistic cells!**



Large & Rapid Expansion Capabilities

PeproGrow™ hMSC Medium has been shown to support robust expansions of MSCs with an average maximum expansion of 36.33 ± 9.49 population doublings, or 86,381,301,347-fold. Average expansion rates of 0.71 ± 0.11 doublings/day (doubling time of 34.33 in ± 4.72 hours) have been seen in over a dozen cell lines. Lower passages have frequently shown much faster rates, many of which double in under 24 hours.

**Growth Rates of Multiple Lines of Mesenchymal Cell Lines
in PeproGrow™ hMSC Medium**

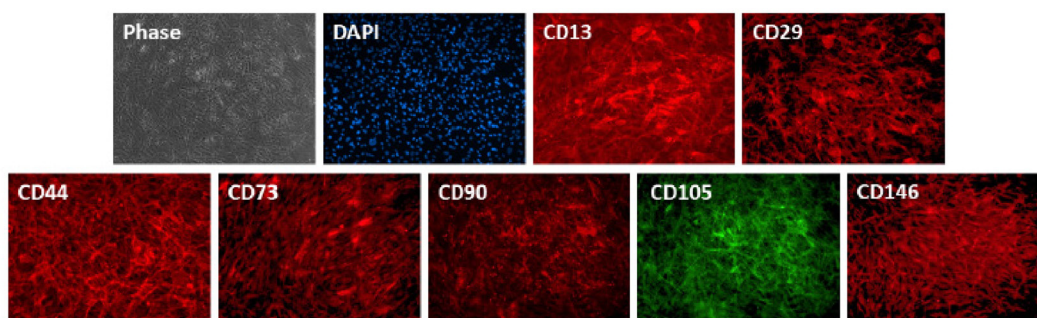


	Days	Approximate Fold Expansion
Cell Line1	6	8
	15	209
	21	4,466
	29	146,206
	37	3,746,557
	46	61,048,822
	70	1,058,631,049
Cell Line 2	6	17
	15	398
	22	13,761
	28	392,168
	37	13,148,549
	51	337,754,279
	58	8,756,810,141
	66	200,580,972,472
	77	3,178,593,514,724
Cell Line 3	6	10
	15	141
	22	3,034
	28	73,996
	37	1,746,504
	46	33,529,876
	70	436,764,936
Cell Line 4	3	3
	7	146
	15	3,215
	21	73,655
	29	1,116,674
	35	29,354,997
	42	474,140,396
	49	15,726,433,432
	56	470,140,104,517
	66	13,276,291,702,378
	81	197,642,144,229,559

Retention of Immunophenotypes

The ability to expand represents only a part of what is required to create and maintain robust stocks of hMSCs. The ability to retain specific immunophenotypes in the undifferentiated state, the ability to differentiate into specific differentiated phenotypes, and the ability to secrete cytokines and growth factors are also major requirements. All of these aspects of hMSC biology have been documented in multiple cell lines. As shown below, **PeproGrow™ hMSC Medium** supports solid retention of immunophenotypes and characteristic morphology across multiple cell lines.

	Population Doublings	Fold Expansion	Positive Markers					Negative Markers		
			CD13	CD29	CD44	CD73	CD90	CD31	CD34	CD45
Cell Line 1	1.570	2.969	+	+	+	+	+	-	-	-
	5.997	63.867	+	+	+	+	+	-	-	-
	10.926	1,945.601	+	+	+	+	+	-	-	-
	15.626	50,570.200	+	+	+	+	+	-	-	-
	20.088	1,114,527.000	+	+	+	+	+	-	-	-
Cell Line 2	1.797	3.475	+	+	+	+	+	-	-	-
	7.018	129.607	+	+	+	+	+	-	-	-
	11.894	3,805.842	+	+	+	+	+	-	-	-
	16.832	116,664.007	+	+	+	+	+	-	-	-
	21.506	2,978,180.987	+	+	+	+	+	-	-	-
Cell Line 3	1.630	3.095	+	+	+	+	+	-	-	-
	6.621	98.428	+	+	+	+	+	-	-	-
	11.815	3,603.043	+	+	+	+	+	-	-	-
	15.978	64,544.207	+	+	+	+	+	-	-	-
	20.045	1,081,798.221	+	+	+	+	+	-	-	-
Cell Line 4	2.839	7.155	+	+	+	+	+	-	-	-
	8.129	279.945	+	+	+	+	+	-	-	-
	13.143	9,045.597	+	+	+	+	+	-	-	-
	18.257	313,259.774	+	+	+	+	+	-	-	-
	23.029	8,558,935.827	+	+	+	+	+	-	-	-
Cell Line 5	2.151	4.441	+	+	+	+	+	-	-	-
	7.843	229.603	+	+	+	+	+	-	-	-
	13.255	9,775.806	+	+	+	+	+	-	-	-
	18.598	396,785.561	+	+	+	+	+	-	-	-
	23.763	14,235,600.345	+	+	+	+	+	-	-	-

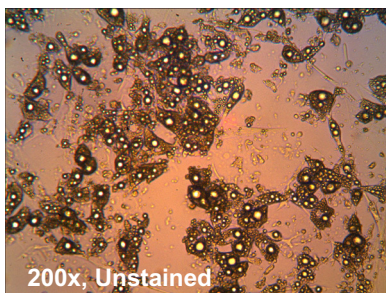


Maintaining Multipotency

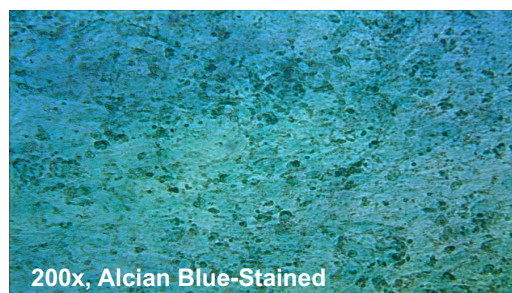
An essential trait of hMSCs, is the ability to successfully differentiate into multiple cell types with specialized functions, including fat storage (adipocytes), cartilage production (chondrocytes), bone production (osteocytes), and muscle production (myocytes). This trait, referred to as multipotency, is a primary hallmark of hMSCs and its degradation is often a major limitation in long-term culture. When expanded in PeproGrow™ hMSC Medium and differentiated according to common protocols, all cell lines tested were shown to maintain full multipotency past 20 population doublings (more than 1,000,000-fold expansion); some retaining full multipotency up to 35 population doublings (more than 34,000,000,000-fold expansion).

Cells were grown on fibronectin-coated plates differentiated for 3-4 weeks, then fixed with 4% paraformaldehyde and stained with Oil Red (adipocytes), Alcian Blue (chondrocytes), or Alizarin Red (osteocytes).

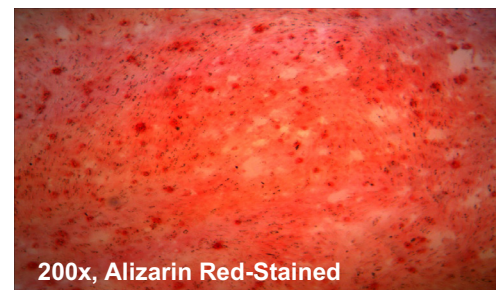
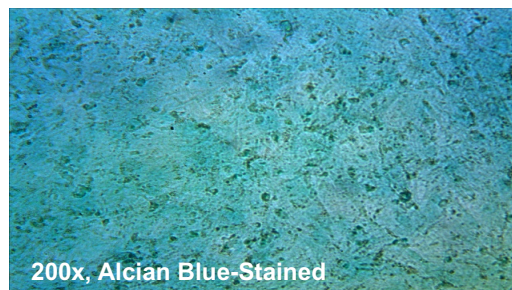
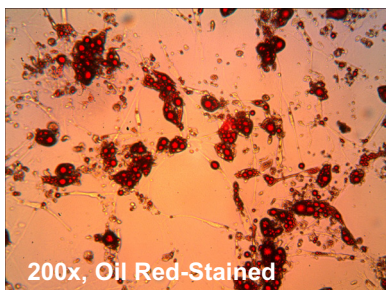
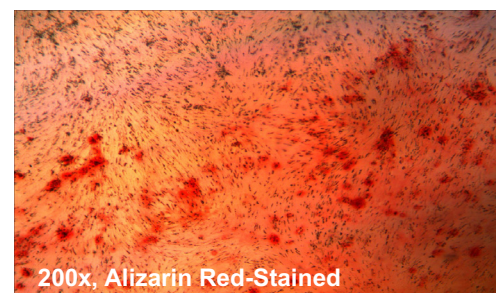
Adipogenesis:



Chondrogenesis:



Osteogenesis:



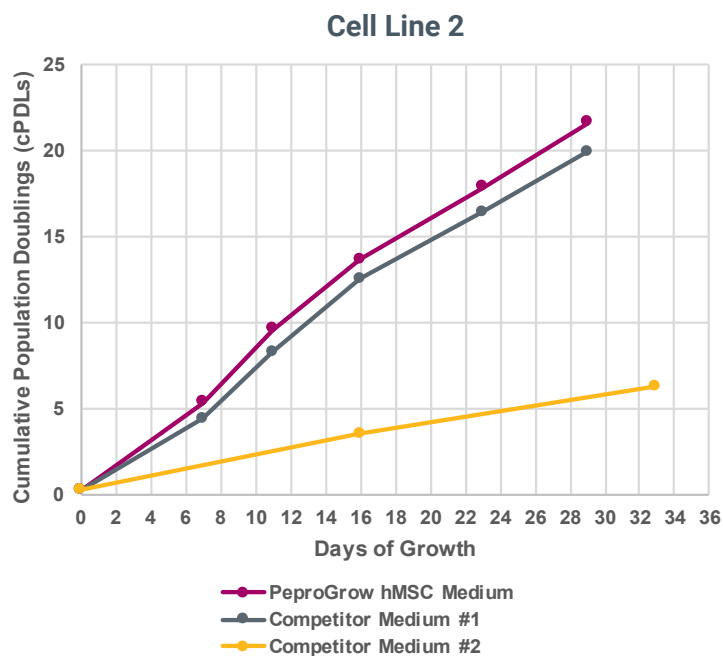
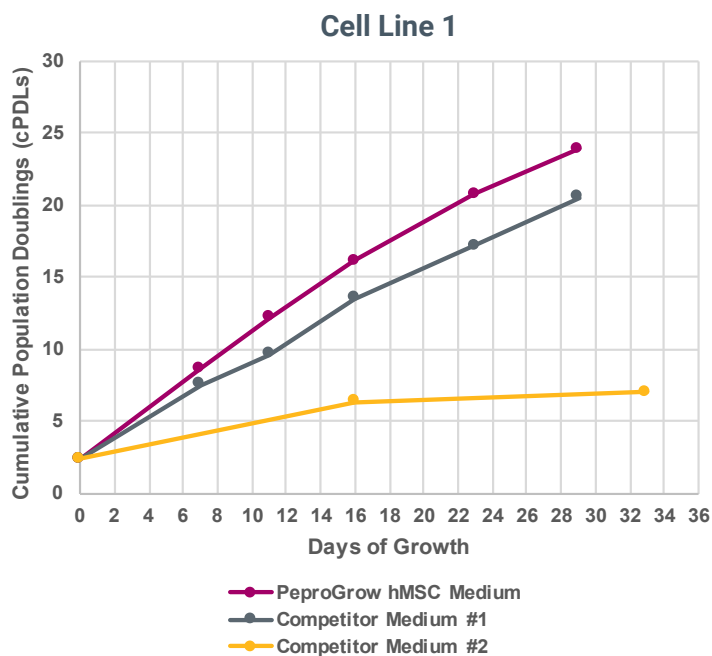
Secretion Profiles of Cytokines, Chemokines, and Growth Factors

PeproGrow™ hMSC Medium has been shown to support the normal physiological functions of MSCs including the secretions of a variety of growth factors, cytokines and chemokines, all of which are known to regulate normal regenerative, restorative, and immunomodulatory functions. Cells were switched to a mitogen-free basal medium, and cells were allowed to secrete into the medium for 96 hours. At the end of that time, samples of the conditioned media were sterile-filtered and subjected to multiplex protein analysis using a Luminex® instrument. A select panel of chemical analytes was selected, and samples were run in triplicate. Cell lines expanded in PeproGrow™ hMSC Medium demonstrated robust secretion of cytokines, chemokines and growth factors, many of which are associated with hMSC controlled repair, regeneration and immunomodulation. The presence of such secretomes indicate the physiological relevancy of the expanded cells.

After expansion, cells were switched to a mitogen-free basal medium and allowed to secrete for 96 hours. Samples of conditioned media was then sterile-filtered and protein secretion was determined via multiplex protein analysis (Luminex).

Secreted Factor	Cell Line 1	Cell Line 2	Cell Line 3	Cell Line 4	Cell Line 5	Cell Line 6
EGF	✓	✓	✓	✓	✓	✓
FGF2	✓	✓	✓		✓	✓
VEGF	✓	✓	✓	✓	✓	✓
PDGF-AB/BB	✓	✓	✓	✓	✓	✓
TGFβ1	✓	✓	✓	✓	✓	✓
TGFβ2	✓	✓	✓	✓	✓	✓
G-CSF	✓	✓	✓	✓	✓	✓
GM-CSF	✓	✓	✓	✓	✓	✓
GRO	✓	✓	✓	✓	✓	✓
Flt-3L	✓	✓	✓	✓	✓	✓
MCP-1α	✓	✓	✓	✓	✓	✓
MCP-3	✓	✓	✓	✓	✓	✓
MIP-1α	✓	✓	✓		✓	✓
IL-1Ra	✓	✓	✓	✓	✓	✓
IL-6	✓	✓	✓	✓	✓	✓
IL-8	✓	✓	✓	✓	✓	✓
IL-10	✓	✓	✓		✓	

Comparative Growth of PeproGrow™ hMSC Medium vs Competitor Products



Kit/Components

Catalog Number:

Size:

PeproGrow hMSC Medium Kit

XF-HMSC-500

500mL

PeproGrow hMSC Basal Medium

BM-XF-HMSC-500

500mL

PeproGrow hMSC Growth Factor Supplement

GF-XF-HMSC-500

500mL

Notes



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