

MesenPure™

For Enriched Mouse MSC Cultures

Highly Enriched Mouse MSC Cultures Without the Wait

It is difficult to establish cultures of mouse mesenchymal stem and progenitor cells (MSCs) both due to their rarity in bone marrow (BM) and compact bone (CB), and because these cultures often contain a high proportion of hematopoietic cells.¹⁻³ Several passaging steps and frequent medium changes are routinely employed to deplete the unwanted cells and enrich for MSCs in culture.^{4,5} These protocols usually take weeks, often leading to a population of cells that is quite different from the MSCs initially observed in culture. Observed changes include a loss of cell surface markers, senescence, and a decrease in the homing ability and differentiation potential of the MSCs.⁶⁻⁸

The **MesenCult™ Proliferation Kit with MesenPure™ (Mouse)** enriches for mouse MSCs in culture without serial passaging and frequent medium changes. Primary MSCs exposed to MesenPure™ appear homogeneous and mostly devoid of hematopoietic cells as early as passage 0 (Figure 1). Cultures also contain increased numbers of MSCs that display more robust differentiation.

Advantages of MesenPure™

SAVES TIME. Fast enrichment of mouse MSC cultures without serial passaging.

HIGH-PERFORMANCE CULTURES. Homogeneous mouse MSC cultures display robust self-renewal and differentiation potential.

VERSATILE. Optimized for use with mouse bone marrow- and compact bone-derived MSCs and MEFs.

EASY TO USE. Simply add MesenPure™ to complete MesenCult™ medium just prior to use.

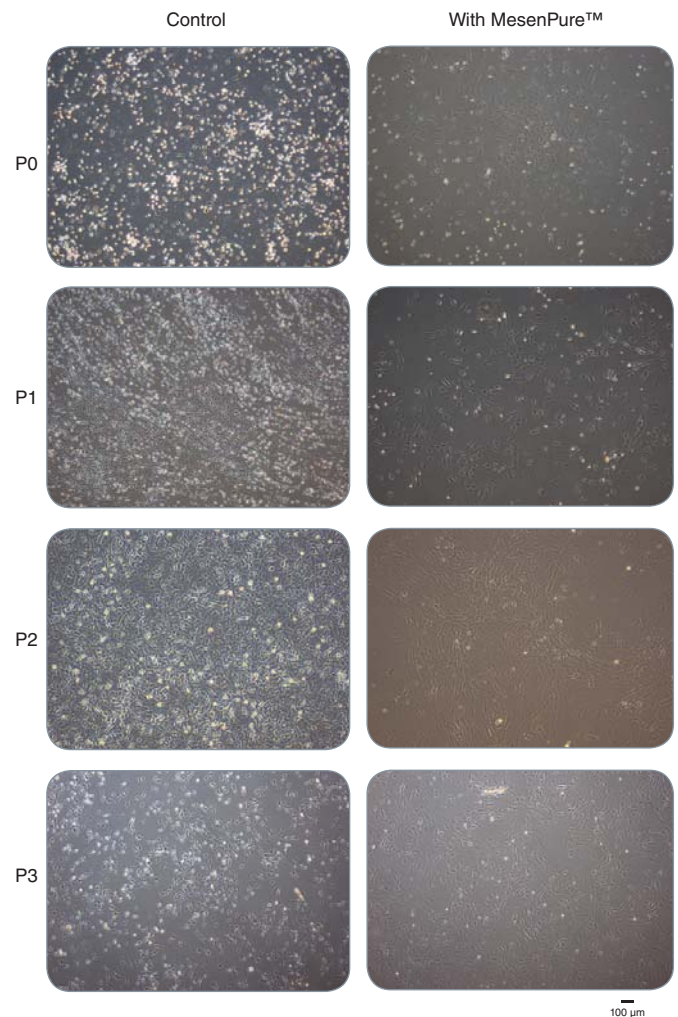


Figure 1. MSC cultures exposed to MesenPure™ appear homogeneous and mostly devoid of hematopoietic cells as early as passage 0 (P0).

Primary BM-derived MSCs were cultured in complete MesenCult™ medium without (Control) or with MesenPure™. In control conditions, hematopoietic cells remained in high numbers as late as P2, with many hematopoietic cells still present at P3 (left panels). In MesenPure™-exposed cultures, low numbers of hematopoietic cells were observed as early as P0, with subsequent passages reducing the number further (right panels).

Reduce Hematopoietic Cells in MSC Cultures

Primary mouse MSC cultures are inherently heterogeneous and contain a large proportion of unwanted hematopoietic cells, viewed as small, round, highly refractile cells under phase contrast optics. Following standard culture protocols, high numbers of hematopoietic cells persist in BM- (Figure 1, left panels) and CB-derived (data not shown) MSC cultures through two or three passages. Including MesenPure™ in the culture medium significantly reduces the number of unwanted hematopoietic cells, generating sufficiently enriched MSC cultures for experimental use as early as P0 (Figure 1, right panels).

Flow cytometry was used to confirm the results observed by light microscopy visualization of the cultures. Primary BM-derived MSCs were cultured for 14 days in complete MesenCult™ medium with or without MesenPure™ prior to flow cytometry analyses being performed. The cell surface marker CD45 is expressed by hematopoietic cells and not by MSCs. In control conditions, only ~9% of the cells were CD45⁺, whereas MesenPure™-exposed cultures contained ~70% CD45⁺ cells (Figure 2, top panels). Further analysis of the CD45⁺ population revealed ~75% of the control cells and ~96% of the MesenPure™-exposed cells co-expressed CD29 and Sca1, suggesting they were MSCs (Figure 2, bottom panels). Similar results were obtained with CB-derived MSCs (data not shown).

Increase the Number of MSCs in Culture

To determine if MesenPure™ affected the total number of MSCs observed in culture, cell counts were also performed. Reduced numbers of total cells were observed in MesenPure™-exposed cultures (Table 1), but relating the percentage of CD45⁺ cells to the total number of cells suggested that similar numbers of CD45⁺ cells were present in both culture conditions. As the percentage of CD45⁺/CD29⁺/Sca1⁺ cells was much higher in the MesenPure™-exposed culture, the total number of MSCs present in the MesenPure™-exposed culture was estimated to be 0.25-fold higher. Taken together, these results suggest that MesenPure™ acts to deplete hematopoietic cells from MSC cultures while increasing their proliferation, thereby enriching for MSCs.

Addition of MesenPure™ to CFU-F cultures of BM- (Figure 3) or CB-derived (data not shown) MSCs reduces the number of hematopoietic cells and results in more homogenous MSC colonies, making identification and quantification of CFU-F simpler. Including MesenPure™ in the culture medium also leads to an increase in the number of CFU-F observed, independent of the number of cells initially plated (Table 2). The increase in the number of CFU-F observed in cultures exposed to MesenPure™ suggests that MesenPure™ is capable of increasing the number of MSCs in culture.

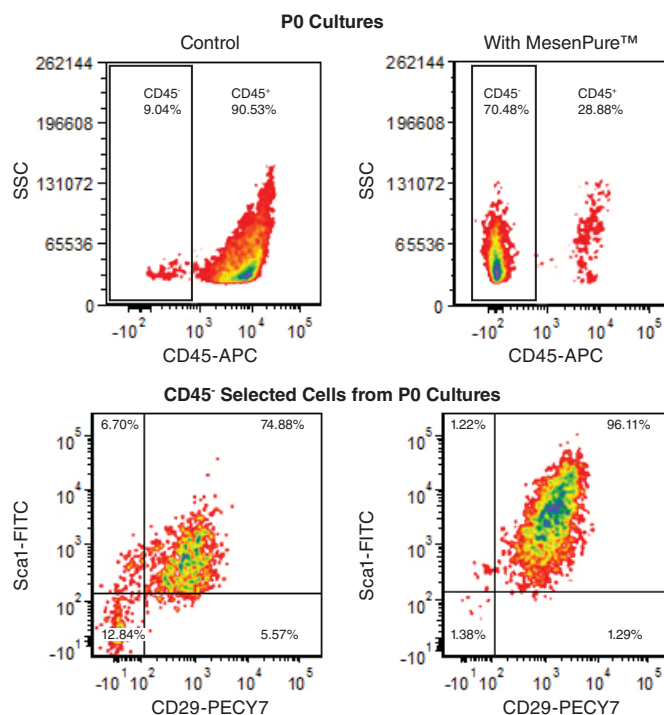


Figure 2. Flow cytometry analysis of P0 MSC culture exposed to MesenPure™ demonstrates significant enrichment of CD45⁺/CD29⁺/Sca1⁺ cells.

Primary BM-derived MSCs were cultured for 14 days in complete MesenCult™ medium without (Control) or with MesenPure™ prior to flow cytometry analysis being performed. ~9% of the control culture and ~70% of the MesenPure™-exposed culture was CD45⁺. Analysis of the CD45⁺ population revealed ~75% of the control cells and ~96% of the MesenPure™-exposed cells co-expressed CD29 and Sca1.

		PERCENT	
	Total Cells	CD45 ⁺	CD45 ⁺ /CD29 ⁺ /Sca1 ⁺
Control	70.0 × 10 ⁵	9.04	74.88
With MesenPure™	9.44 × 10 ⁵	70.48	96.11
		NUMBER OF CELLS	
	Total Cells	CD45 ⁺	CD45 ⁺ /CD29 ⁺ /Sca1 ⁺
Control	70.0 × 10 ⁵	632,800 (1.00)	473,841 (0.75)
With MesenPure™	9.44 × 10 ⁵	665,331 (1.05)	639,450 (1.01)

Table 1. Exposure of MSC cultures to MesenPure™ leads to enrichment of CD45⁺ cells and may increase expansion of CD45⁺/CD29⁺/Sca1⁺ cells.

Flow cytometry analysis of BM-derived MSC cultures exposed to MesenPure™ shows a marginal increase in the number of CD45⁺ cells, but a 0.25-fold increase in CD45⁺/CD29⁺/Sca1⁺ cells compared to cultures without MesenPure™. The ratio of CD45⁺ cells and CD45⁺/CD29⁺/Sca1⁺ cells was applied to the total number of cells to obtain the actual number of cells in culture. Fold-induction from control cultures is reported in parenthesis.

Highly Enriched MSC Cultures Without the Wait

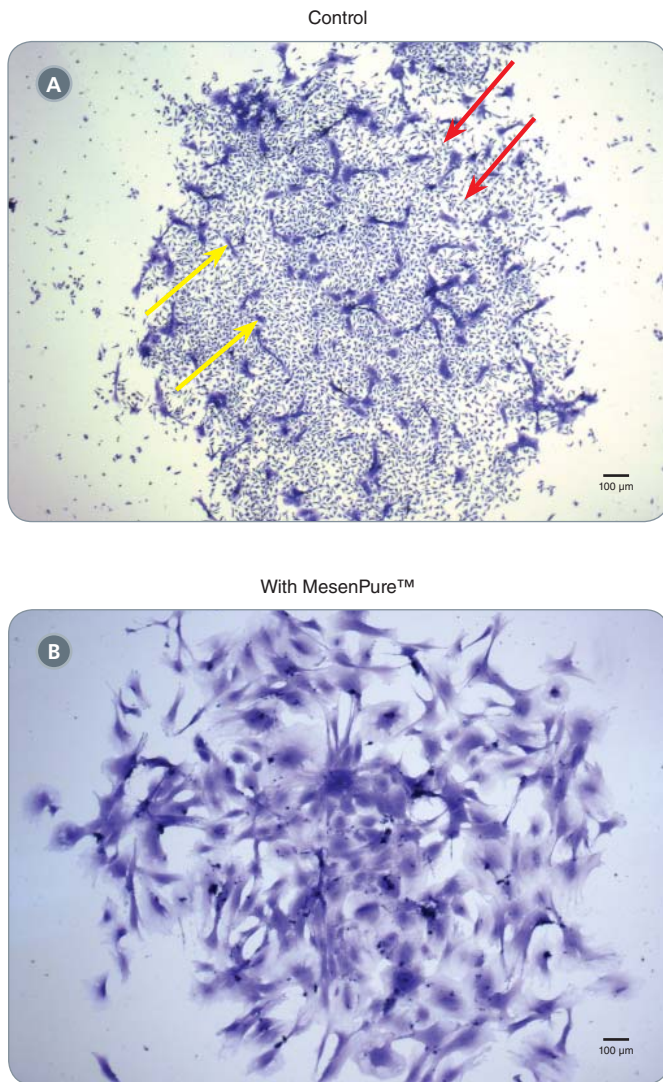


Figure 3. CFU-F assay cultures exposed to MesenPure™ are homogeneous and devoid of hematopoietic cells.

CFU-F assays were performed with primary BM-derived MSCs. Cells were cultured in complete MesenCult™ medium without (Control) or with MesenPure™ for 14 days, fixed, and stained with toluidine blue. (A) In the control condition, a large number of hematopoietic cells (red arrows) are present and surround the MSCs (yellow arrows). (B) Cultures exposed to MesenPure™ are homogeneous and devoid of hematopoietic cells.

BM-MSCs

		NUMBER OF CFU-F		
Assay #	# of Cells Plated	Control	With MesenPure™	% Increase
1	2.5 × 10 ⁵	82	103	26
	5.0 × 10 ⁵	140	201	44
	10.0 × 10 ⁵	180	227	26
	Total MSC Colonies Across All Wells	402	531	32
2	2.5 × 10 ⁵	4	3	0
	5.0 × 10 ⁵	11	23	109
	10.0 × 10 ⁵	28	34	21
	Total MSC Colonies Across All Wells	43	60	40
3	2.5 × 10 ⁵	3	5	67
	5.0 × 10 ⁵	10	17	70
	10.0 × 10 ⁵	40	60	50
	Total MSC Colonies Across All Wells	53	82	55

CB-MSCs

		NUMBER OF CFU-F		
Assay #	# of Cells Plated	Control	With MesenPure™	% Increase
1	0.5 × 10 ⁵	27	46	70
	1.0 × 10 ⁵	51	77	51
	2.5 × 10 ⁵	102	133	30
	Total MSC Colonies Across All Wells	180	256	42
2	2.5 × 10 ⁵	12	15	25
	5.0 × 10 ⁵	33	36	9
	10.0 × 10 ⁵	45	83	84
	Total MSC Colonies Across All Wells	90	134	49
3	2.5 × 10 ⁵	28	35	25
	5.0 × 10 ⁵	69	89	29
	10.0 × 10 ⁵	80	147	84
	Total MSC Colonies Across All Wells	177	271	53

Table 2. In CFU-F assays, exposure of primary cultures of BM- or CB-derived MSCs to MesenPure™ led to an overall minimum 20% increase in the number of MSC colonies observed, independent of the number of cells initially plated.

MesenPure™

Highly Enriched MSC Cultures Without the Wait

MSCs Maintain Robust Multi-Lineage Differentiation Potential

To ensure MesenPure™-treated MSCs retain multi-lineage differentiation potential, cultures were maintained in complete MesenCult™ Medium without (Control) or with MesenPure™ for two passages and then plated at different densities for osteogenic and adipogenic differentiation experiments. MSC cultures were maintained for one passage before being plated for chondrogenic differentiation experiments. Cultures exposed to MesenPure™ displayed much stronger osteogenic (Figure 4A and 4B) and adipogenic differentiation (Figure 4B) at lower cell densities than MSCs cultured in control conditions. Strong chondrogenic differentiation was also observed with MesenPure™-exposed MSCs (Figure 4B). The improved differentiation ability of the MesenPure™-exposed cultures at low cell densities may be due to the enrichment of MSCs compared to control conditions.

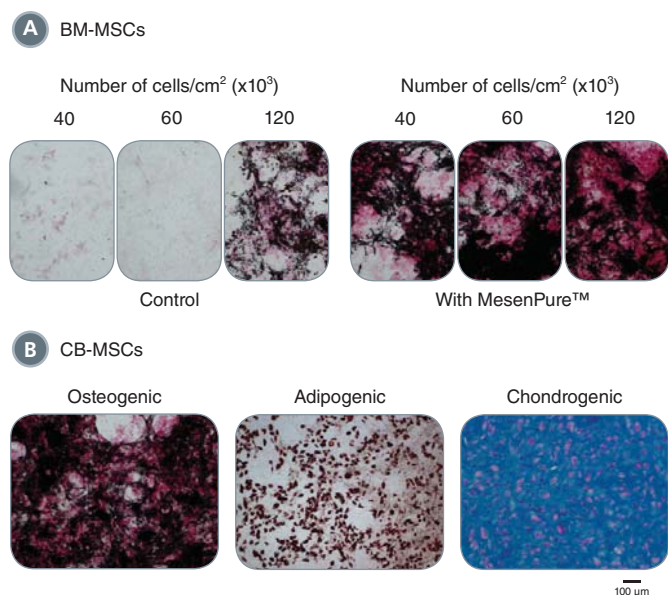


Figure 4. MSCs exposed to MesenPure™ require fewer cells to demonstrate robust differentiation.

(A) BM-derived MSCs initially cultured in complete MesenCult™ medium without (Control) or with MesenPure™ were plated at three different densities and then differentiated using MesenCult™ Osteogenic Stimulatory Kit (Mouse; Catalog #05504). Differentiation cultures were fixed and stained for Alkaline Phosphatase (ALP) activity (red areas) and mineralization (black areas). In control cultures, modest differentiation into the osteogenic lineage was only observed when cultures were seeded at the highest density. In MesenPure™-exposed cultures, robust osteogenic differentiation was observed at all seeding densities. (B) 4.0×10^4 cells/cm² of P2 CB-derived MSCs were used in osteogenic and adipogenic differentiation. 5.0×10^5 cells of P1 CB-derived MSCs were used for chondrogenic differentiation. MesenPure™-exposed cultures displayed strong osteogenic, adipogenic and chondrogenic differentiation.

New



Figure 5. MesenCult™ Proliferation Kit with MesenPure™ (Mouse)



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Ordering Information

PRODUCT	QUANTITY	CATALOG #
MesenCult™ Proliferation Kit with MesenPure™ (Mouse)	1 kit*	05512

*Each kit contains MesenCult™ MSC Basal Medium (Mouse; 400 mL), MesenCult™ Mesenchymal Stem Cell Stimulatory Supplements (Mouse; 100 mL) and MesenPure™ (0.5 mL)

References

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