Stasis™ Fetal Bovine Serum

Catalog Number: 100-125 **Lot Number: A65H02K**

FBS Characterization: Assessing Fetal Bovine Serum on Mesenchymal stem cell proliferation, differentiation, and growth over time.

Objective

To evaluate various lots of Fetal Bovine Serum (FBS) on the proliferation and differentiation of human bone marrow derived mesenchymal stem cells (MSC).

Overview

MSC culture techniques were used to assay the effects of several lots of FBS. We tested the FBS in its capacity to promote proliferation over time and differentiation of MSCs.

Test Article

Test Article Information

Identity:	Human bone marrow-derived MSCs
Passage Number:	2
Donor Number:	MSC 68
Viral Vector:	NA
MOI:	NA
Transduction Date:	NA
Storage Conditions:	Cryopreserved cells were kept at or below -80°C. Cells were cultured according to human MSC culture protocol.

Experimental Design

Cell Differentiation

For osteogenic differentiation, MSCs were cultured in triplicate for 15 days in either control media or MEM--a containing 10% fetal bovine serum (FBS), 1× L-glutamine, 0.2 mM ascorbic acid, 0.1 µM dexamethasone, and 10 mM β-glycerophosphate (i.e. osteogenic media), with medium changes every 3-4 days. After X days, cells were washed with PBS, fixed using formalin, washed, and stained with Alizarin Red S for 30 minutes. Wells were then washed and documented photographically. To quantify osteogenesis, stained wells were incubated with 10% acetic acid for 30 minutes. After incubation, supernatants were collected, and absorbance measured at 405nm. Values shown below indicate absorbance of cells in osteogenic media minus cells in control media (background).

For adipogenic differentiation, MSC were cultured for 12 days in MEM-a containing 10% FBS, 1× L-glutamine, 0.5 mM isobutylmethyxanthine, 50 µM indomethacin, and 0.5 µM dexamethosone with medium changes every 3-4 days. Then, cells were fixed as above, stained with Oil Red O and documented photographically. To quantify adipogenesis, cells stained with Oil Red O were treated with 4% Tween-20 in isopropanol for 5 minutes. Absorbance of supernatants were measured in a plate reader at 490nm. Values shown indicate absorbance of cells in adipogenic media minus cells in control media (background).

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Cell Proliferation

MSCs were cultured using mem-a supplemented with 10% FBS, accordingly. In brief, MSCs (2000 of cells/well) were plated into 24-well plates. To assess proliferation, we employed CellTiter96 Non-radioactive Cell Proliferation Assay (Promega) following manufacturer's protocol. At each timepoint, culture plates were stored at 4°C until the time course was complete; at this time the plates were processed and analyzed on the plate reader at 490nm-650nm.

Cell Proliferation over multiple passages

MSCs were cultured using mem-a supplemented with 10% FBS, accordingly. In brief, we plated 3,800 MSCs into a 12-well plate. Upon reaching 70% confluency, the cells were lifted and counted manually on a hemocytometer. This was repeated for 3 cell passages.

Results

Cell Differentiation:

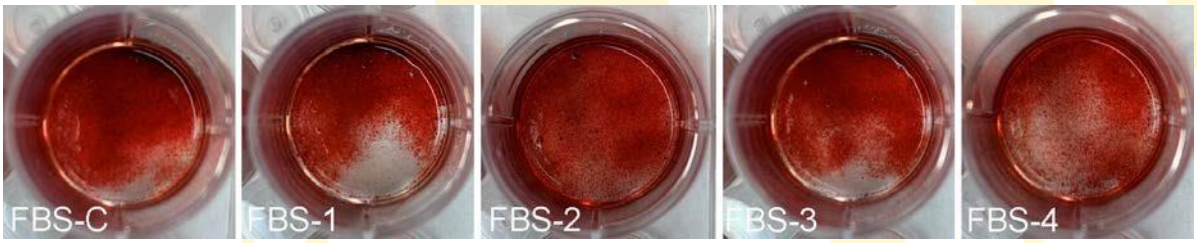

Figure1A

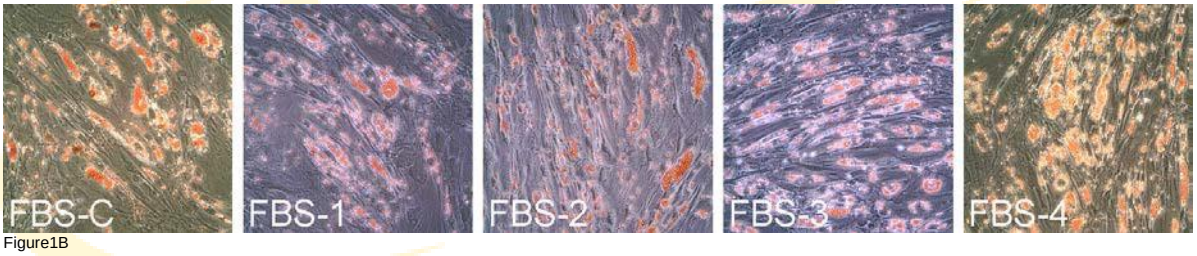

Figure1B

Figure 1. Effect of different lots of FBS on osteogenic and adipogenic differentiation of MSCs. Figure 1A shows representative wells of MSCs after osteogenesis, as assessed by Alizarin Red Staining after 15 days in culture in osteogenic media. Figure 1B shows adipogenesis that was assessed after 12 days in adipogenic media by formation of adipocytes using Oil Red O. Representative images (original magnification 10x) are shown. Visual inspection suggests that differentiation of cells performed very similarly with the different lots of FBS.

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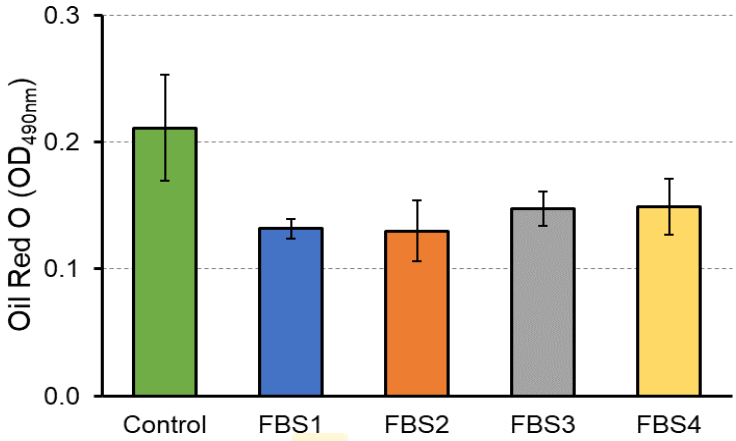

Figure 2. Quantification of adipogenic differentiation of MSC using various lots of FBS. After 12 days cells were stained with Oil Red O (shown above) and treated with 4% tween in isopropanol. Absorbance was read on a plate reader at 490nm with three triplicates and one control well with no differentiation media for each condition. The "Control" media displayed slightly higher differentiation potential, while all other samples displayed very similar levels.

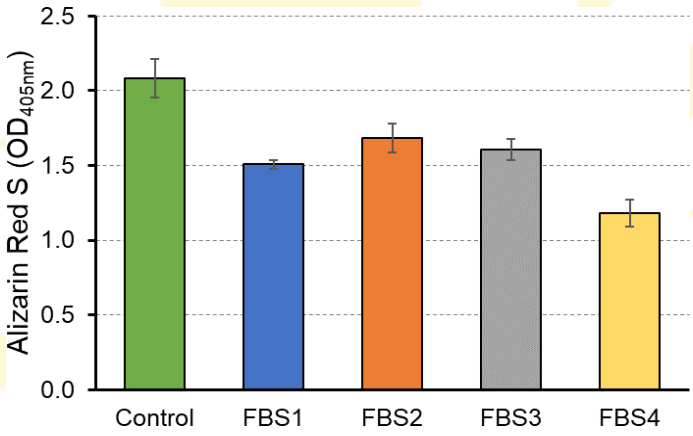

Figure 3. Quantification of osteogenic differentiation of MSC, using various lots of FBS. After 12 days, cells were stained with Oil Red R and treated with 4% tween in isopropanol. Absorbance was read on a plate reader at 405nm with three triplicates and three control wells with no differentiation media for each condition. Also, here, osteogenic media with "Control" FBS displayed slightly higher differentiation, while all other samples displayed similar levels of osteogenesis. These results are therefore consistent with the adipogenesis assay, showing that the control had slightly higher differentiation potential, while all Gemini FBS performed very similarly to each other.

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Cell proliferation:

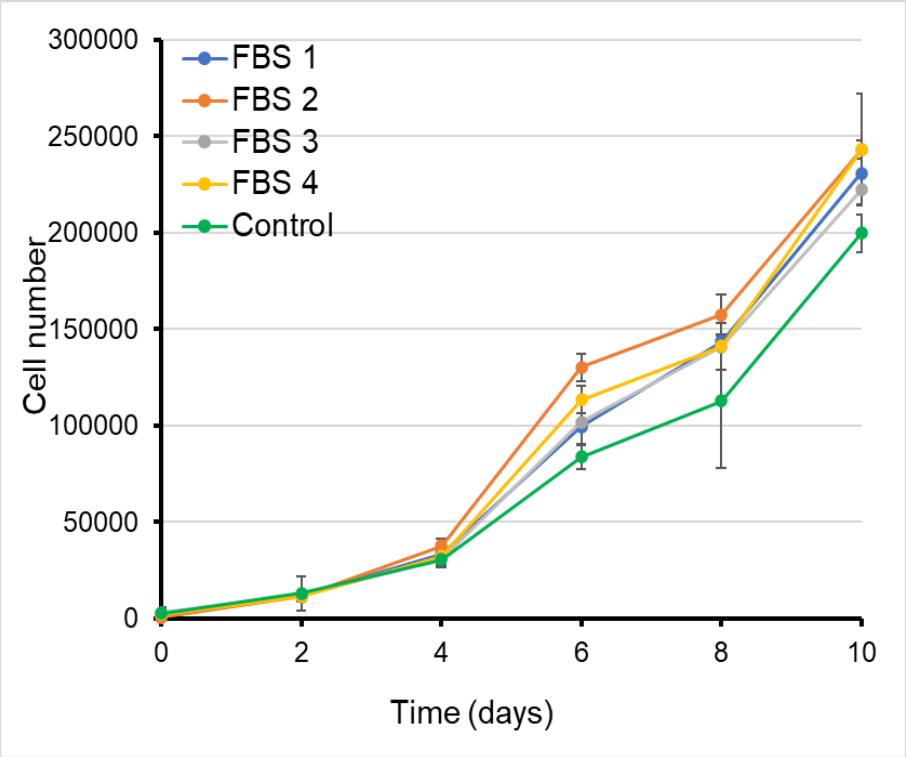

Figure 4. Proliferation rates of MSC in various FBS over time. After 0, 2, 4, 6, 8, and 10 days in culture, plates were analyzed using CellTiter96 nonradioactive cell proliferation assay (Promega) and absorbance was read on a plate reader. All samples performed very similarly in the growth curve analysis, where the “control” FBS performed the poorest as compared to all other samples. All other samples performed similarly well.

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Cell counts/proliferation over multiple passages:

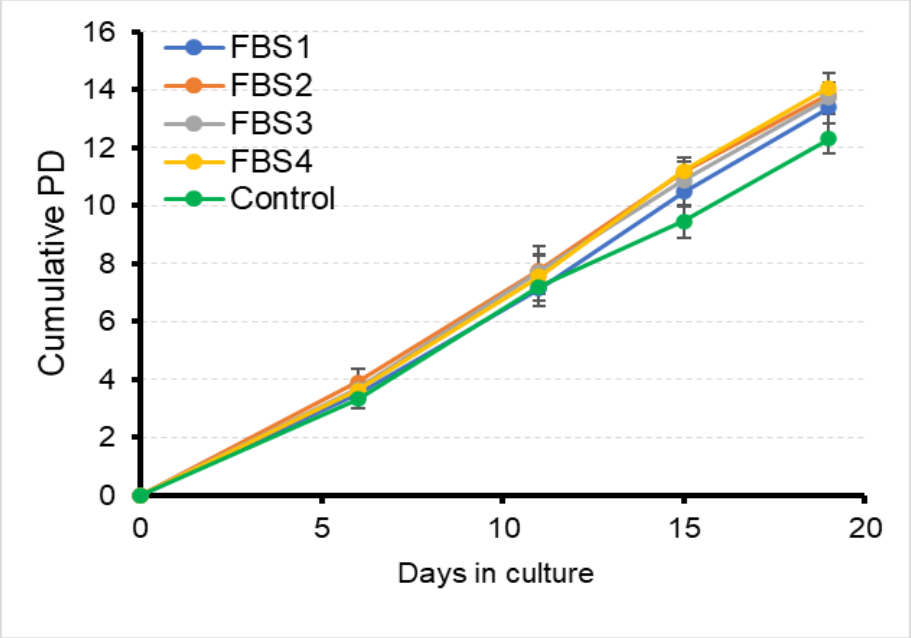

Figure 5. Doubling time of MSC using various FBS over passages. Cells were counted manually using a hemocytometer after each passage, for a total of 4 passages. To calculate the cumulative population doubling (PD), we used the following equation: $t_n = 3{,}800*2^n$, where t_n represents the number of cells at time "t", and n represents the population doubling. Results were consistent with the proliferation curves (Figure 4), showing that all samples performed similarly, with Control FBS performing slightly worse compared to all samples.

Sample Key	
Gemini 1	BenchMark A16H74K
Gemini 2	GemCell A28H74L
Gemini 3	BenchMark A33H00L
Gemini 4	Stasis FBS A65H02K
Control	House FBS