

Stem Cells

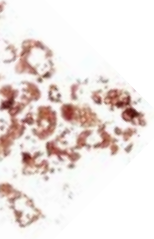


# Gain time and improve performance

MSC-Qualified Fetal Bovine Serum



 **invitrogen**™



## Stem Cells

We've done the work for you—now you don't have to test multiple fetal bovine serum (FBS) lots

- Attain enhanced mesenchymal stem cell (MSC) clonal efficiency
- Improve expansion of MSCs
- Obtain sustainable MSC differentiation

### Simplify your MSC culture

FBS is a major component needed for the culture of human mesenchymal stem cells. However, there are many unknown elements in FBS, such as signaling molecules, apoptotic factors, and nutrients. The variable concentration of these components can cause lot-to-lot variation, which means that some FBS lots do not support MSC culture. For that reason, extensive and time-consuming pretesting is required. Our MSC-Qualified FBS eliminates the need for you to test multiple FBS lots to identify the optimal one for MSC research.

### Greater clonal efficiency than the competition

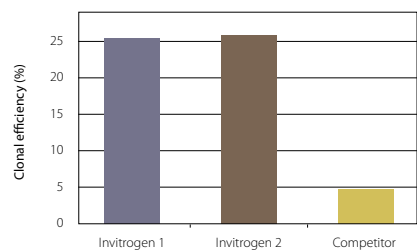
The ability of individual MSCs to form colonies in culture (clonal efficiency) is very important in MSC research and has been shown to be highly dependent on the choice of FBS used in the medium. Clonal efficiency is measured using the CFU-f (colony-forming unit fibroblast) assay, which determines the ratio of the number of colonies formed to the initial number of plated cells. Figure 1 shows the superior clonal efficiency obtained using Invitrogen's MSC-Qualified FBS compared to a competitor's product.

# Greater expansion than the competition

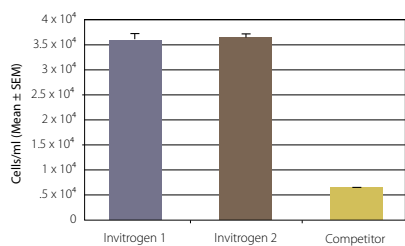
Culture-expanded MSCs are used in a growing list of research applications including cell differentiation, gene expression, cell signaling, tissue remodeling, and tissue engineering. They are also being investigated for use in potential cell-based therapies in the field of regenerative medicine. The ability to expand cultures to higher densities or at faster rates can significantly reduce the cost of research and therapy. Invitrogen's MSC-Qualified FBS can significantly improve MSC expansion characteristics (Figure 2).

## Sustainable differentiation

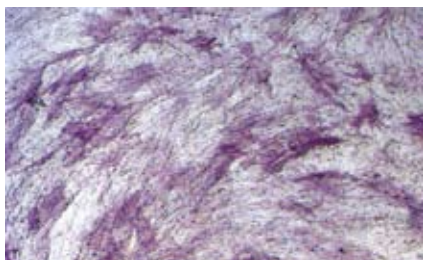
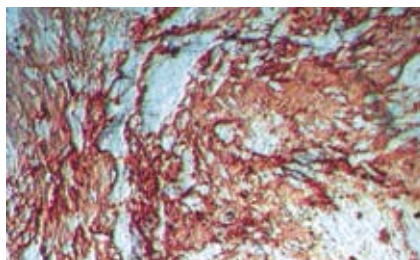
MSCs are multipotent, self-renewing, mesodermal-origin cells that can differentiate into a growing list of differentiated cell types. Traditionally, MSCs have been defined by their ability to differentiate into the three lineages of bone, cartilage, and fat. MSCs were shown to maintain their ability to differentiate into bone (Figure 3), chondrocytes (Figure 4), and adipocytes (Figure 5) when cultured using Invitrogen's MSC-Qualified FBS.



**Figure 1—Effect of FBS source on MSC clonal efficiency.** Mesenchymal stem cells were isolated from normal human bone marrow mononuclear cells by standard techniques. Early passaged cells were plated into duplicate 100 mm tissue culture dishes at a seeding density of 100 cells per plate in DMEM (low glucose), 4 mM L-glutamine, 5 µg/ml gentamicin, and 10% of the indicated FBS. On day 14 the medium was removed and the plates were rinsed and stained with 0.5% Crystal Violet in methanol for 30 min. Plates were rinsed and dried, and the colonies were counted using a dissection microscope. Only colonies with at least 50 cells were counted. Invitrogen's MSC-Qualified FBS outperformed a competitor's MSC-qualified FBS (P <0.05; Student's t-test).



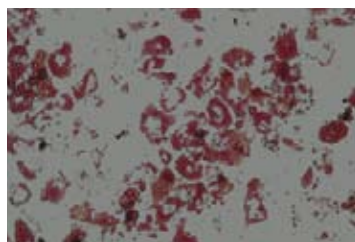
**Figure 2—Effect of FBS source on MSC expansion.** Mesenchymal stem cells were isolated from normal human bone marrow mononuclear cells by standard techniques. Early passage cells were seeded into 24-well plates at a density of 3 x 10<sup>3</sup> cells per well (1.5 x 10<sup>3</sup> cells per cm<sup>2</sup> in 2 ml of medium) in DMEM (low glucose), 4 mM L-glutamine, 5 µg/ml gentamicin, and 10% of the indicated FBS. On day 7 the cells were harvested with TrypLE™ Express trypsin replacement enzyme and counted with a Coulter Counter® Model ZM. Data represent cell count averages for eight replicate wells. Invitrogen's MSC-Qualified FBS outperformed a competitor's MSC-qualified FBS (P <0.05; Student's t-test).

**A****B**

**Figure 3—Histological staining of osteogenic cultures.** MSCs were initiated in DMEM, 10% MSC-Qualified FBS, 4 mM L-glutamine, and 5  $\mu\text{g}/\text{ml}$  gentamicin at a seeding density of  $5 \times 10^3$  cells per  $\text{cm}^2$  in 12-well plates. Two hours after seeding, the medium was changed and supplemented with 100 nM dexamethasone, 10 mM sodium  $\beta$ -glycerophosphate, 50  $\mu\text{M}$  ascorbic acid-2-phosphate, and 10 ng/ml BMP-2. Plates were refed every three to four days. Control wells did not contain bone induction factors. **A.** Plates were stained for alkaline phosphatase on day 14 using commercially available kits. **B.** Plates were stained with Alizarin Red S on day 25 using standard staining techniques.



**Figure 4—Histological staining of chondrogenic cultures.** MSCs were initiated in DMEM, 10% MSC-Qualified FBS, 4 mM L-glutamine, and 5  $\mu\text{g}/\text{ml}$  gentamicin at a seeding density of  $5 \times 10^3$  cells per  $\text{cm}^2$  in a T75 or T160 flask, and grown to 70% confluence. For chondrogenic cultures, cells were resuspended to  $1.6 \times 10^7$  cells per ml. The cell suspension (10  $\mu\text{l}$ ,  $1.6 \times 10^5$  cells) was carefully placed into the center of each well of six-well plates. The plates were incubated at  $37^\circ\text{C}$ , 5%  $\text{CO}_2$  in humidified air for 2 hr to allow the cells to attach. Chondrogenic differentiation medium or control medium was then gently overlaid onto the cells, and the plates were incubated for two weeks with re-feeding twice per week. The chondrogenic medium was additionally supplemented with 10 ng/ml TGF  $\beta$ 1, 50 nM ascorbic acid-2-phosphate, and 6.25  $\mu\text{g}/\text{ml}$  insulin. The resulting pellet-like constructs were stained with Alcian Blue using standard staining methods.



**Figure 5—Histological staining of adipogenic cultures.** MSCs were initiated in DMEM, 10% MSC-Qualified FBS, 4 mM L-glutamine, and 5  $\mu\text{g}/\text{ml}$  gentamicin at a seeding density of  $5 \times 10^3$  cells per  $\text{cm}^2$  in 12-well plates. For adipogenic induction, the medium was supplemented with 0.5 mM isobutyl-methylxanthine, 1  $\mu\text{M}$  dexamethasone, 10  $\mu\text{M}$  insulin, and 200  $\mu\text{M}$  indomethacin. Cultures were re-fed two times per week with a complete change of medium. On day 7, cultures were fixed and stained with Oil Red O using standard staining methods.

Save valuable time. Use Invitrogen’s MSC-Qualified Fetal Bovine Serum in your research.

For additional information and to place your order, please visit [www.invitrogen.com/stemcell](http://www.invitrogen.com/stemcell) and [www.invitrogen.com](http://www.invitrogen.com).

Product	Quantity	Cat. no.
Fetal Bovine Serum, MSC-Qualified (USDA approved)	100 ml	12662-011
	500 ml	12662-029

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