

Less FBS, better performance.

MesenPRO RS™ Medium
The Complete Mesenchymal Stem Cell Culture System





MesenPRO RS™ Medium—a reduced-serum formulation for culture of mesenchymal stem cells (MSCs)

- Consistently improves expansion of MSCs
- Maintains multilineage differentiation capabilities
- Provides gene expression profiles comparable to classical media
- Eliminates the need to spend time and money prequalifying FBS lots

More control over your MSC culture

With advances in the use of stem cells in regenerative medicine, cell-based therapies, and tissue engineering reported almost daily, MSC research is moving at a rapid pace. Currently, MSC research is hindered by the variability introduced by adding 10–20% fetal bovine serum (FBS) to the media used for MSC culture. Although FBS does have a proliferative effect on most cells, it also contains a number of components that are present in variable concentrations from one lot to the next. Some serum components are not even known or characterized. Using relatively large concentrations of inherently variable FBS in current MSC culture media causes inconsistent results and loss in differentiation potential, and leads to wasted time trying to identify the best FBS lots to support MSC culture.

MesenPRO RS™ Medium provides a solution to these problems by reducing the FBS supplementation needed to just 2%, and uses proprietary ingredients that have been optimized for the expansion of MSCs. The result is a cGMP-manufactured medium that gives you the quality and consistency needed to optimize your MSC culture.

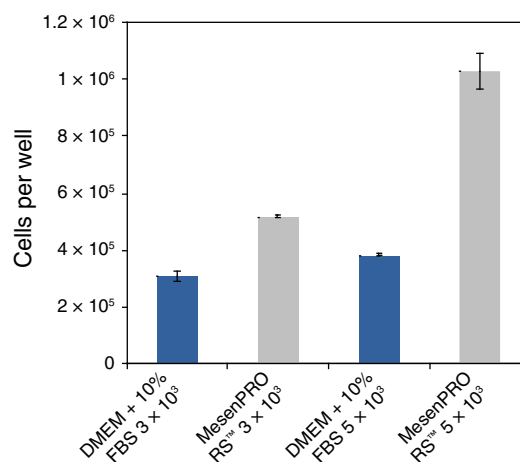


Figure 1—MesenPRO RS™ Medium provides a 69–169% improvement in expansion over six days compared to classical medium. MSCs were isolated from normal human bone marrow mononuclear cells by standard techniques. Early-passage cells were plated into 6-well plates at either 3×10^3 or 5×10^3 cells per cm^2 in DMEM (low glucose) containing 4 mM L-glutamine, 5 $\mu\text{g}/\text{ml}$ gentamicin, and 10% MSC-Qualified FBS, or MesenPRO RS™ Medium containing 2 mM L-glutamine and 5 $\mu\text{g}/\text{ml}$ gentamicin. Cells were incubated at 37°C with 5% CO_2 in humidified air and fed on day 3. On day 6, cells were harvested using TrypLE™ reagent and counted with a Z2™ COULTER COUNTER® particle counter (Beckman Coulter, Inc.). Data represent cell count averages from duplicate wells ($p \leq 0.007$ and $p \leq 0.002$, respectively, by Student's t-test).

MesenPRO RS™ Medium consistently enables superior MSC expansion.

Cell expansion is a key step in any cell culture workflow. Cells expanded in culture are used in a multitude of research applications including cell differentiation, gene expression, cell signaling, tissue remodeling, and tissue engineering. Superior cell expansion is achieved using MesenPRO RS™ Medium compared to expansion using classical medium (DMEM plus 10% MSC-Qualified FBS) (Figure 1).

Product consistency is critical in every cell culture workflow to obtain reproducible expansion of cells. Different lots of MesenPRO RS™ Medium consistently provide a 35–45% improvement in expansion over four days compared to classical medium (Figure 2).

MesenPRO RS™ Medium maintains multipotentiality

Mesenchymal stem cells are multipotent, self-renewing mesodermal-origin cells that can differentiate into several cell types. MSCs

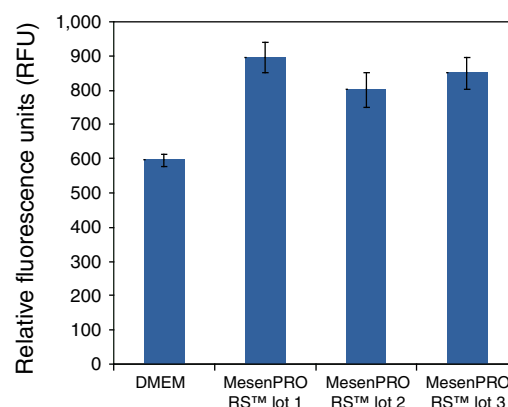


Figure 2—MesenPRO RS™ Medium consistently provides a 35–45% improvement in expansion over four days compared to classical medium. Mesenchymal stem cells were isolated from normal human bone marrow mononuclear cells using standard techniques. Early-passage cells were seeded into 96-well plates at a density of 1,000 cells/well (320 cells/cm² in 200 µl of medium) in either DMEM (low glucose), 4 mM L-glutamine, 5 µg/ml gentamicin, and 10% MSC-Qualified FBS, or in three lots of MesenPRO RS™ Medium, 2 mM L-glutamine, and 5 µg/ml gentamicin. On day 4, cells were assayed using the CyQUANT® NF fluorescent assay, and read on a Gemini EM fluorescent plate reader (Molecular Devices Corp.). Data represent relative fluorescence of labeled nuclei of cells in 18 replicate wells. All three lots of MesenPRO RS™ Medium consistently outperformed the DMEM (n = 12 assays for MesenPRO RS™ and n = 10 for DMEM) (p < 0.01 by Student's t-test). The CyQUANT® NF assay provides a fast, sensitive method for counting cells in a population and measuring proliferation in a microplate format, with a linear detection range of 100–20,000 cells per well for most cell types. The CyQUANT® NF assay uses a cell-permeant DNA-binding dye in combination with a plasma membrane permeabilization reagent for tagging cell nuclei.

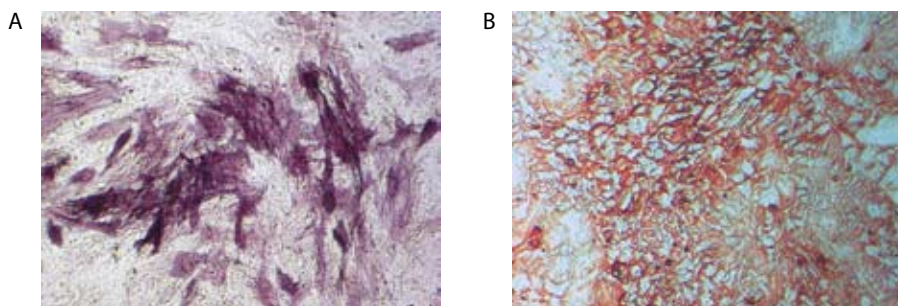


Figure 3—MSCs grown in MesenPRO RS™ Medium can differentiate into the osteogenic lineage. After passaging in MesenPRO RS™ Medium, 2 mM L-glutamine, and 5 µg/ml gentamicin, MSCs were harvested and seeded in DMEM, 10% MSC-Qualified FBS, 2 mM GlutaMAX™ reagent, and 5 µg/ml gentamicin (MSC basal medium) at a seeding density of 5×10^3 cells/cm² in a 12-well plate. The plate was incubated for two hours at 37°C with 5% CO₂ in humidified air. The medium was replaced with fresh osteogenic medium, which consists of MSC basal medium supplemented with 100 nM dexamethasone, 10 mM sodium β-glycerophosphate, 50 µM ascorbic acid-2-phosphate, and 10 ng/ml recombinant human BMP-2. Cells were refed every three to four days. **A.** Cells were stained for osteoblasts with alkaline phosphatase on day 14 using a commercially available kit. **B.** Cells were stained for osteocytes with Alizarin Red S on day 28 using standard staining techniques.

grown in MesenPRO RS™ Medium were able to differentiate into all three lineages traditionally derived from MSCs, including osteogenic (Figure 3), adipogenic (Figure 4), and chondrogenic cells (Figure 5).

MesenPRO RS™ Medium maintains the gene expression profile of MSCs

It is important that a new culture medium does not significantly alter the genetic profile of cells as compared to traditional media. To confirm that MesenPRO RS™ Medium maintains the standard genetic profiles, Illumina BeadArray™ technology was used to compare the gene expression profiles of cells grown in MesenPRO RS™ Medium with those grown in DMEM plus 10% FBS. Similar gene expression is obtained when MSCs are grown in either medium (Figure 6).

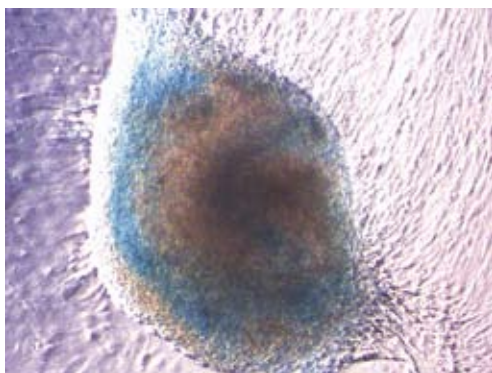


Figure 5—MSCs grown in MesenPRO RS™ Medium can differentiate into the chondrogenic lineage. After passaging in MesenPRO RS™ Medium, 2 mM L-glutamine, and 5 µg/ml gentamicin, MSCs were harvested and resuspended in DMEM, 10% MSC-Qualified FBS, 2 mM GlutaMAX™ reagent, and 5 µg/ml gentamicin (MSC basal medium) at a density of 8×10^6 cells/ml. A cell suspension drop (10 µl) was carefully placed into the center of each well of a 12-well plate. The plate was incubated for two hours at 37°C with 5% CO₂ in humidified air. Chondrogenic medium, which consists of MSC basal medium supplemented with 10 ng/ml recombinant human TGF-β1, 50 nM ascorbic acid-2-phosphate, and 6.25 µg/ml recombinant human insulin, was gently added to the wells. Cells were refed every 3–4 days, and the resulting pellet-like constructs were stained with Alcian Blue on day 14 using standard staining techniques.



Figure 4—MSCs grown in MesenPRO RS™ Medium can differentiate into the adipogenic lineage. After passaging in MesenPRO RS™ Medium, 2 mM L-glutamine, and 5 µg/ml gentamicin, MSCs were harvested and seeded in DMEM, 10% MSC-Qualified FBS, 2 mM GlutaMAX™ reagent, and 5 µg/ml gentamicin (MSC basal medium) at a seeding density of 2×10^4 cells/cm² in a 12-well plate. The plate was incubated for two hours at 37°C with 5% CO₂ in humidified air. The medium was replaced with fresh adipogenic medium, which consists of MSC basal medium supplemented with 1 µM dexamethasone, 0.5 mM isobutylmethylxanthine, 200 µM indomethacin, and 10 µM recombinant human insulin. Cells were refed every 3–4 days, then stained with Oil Red O on day 14 using standard staining techniques.

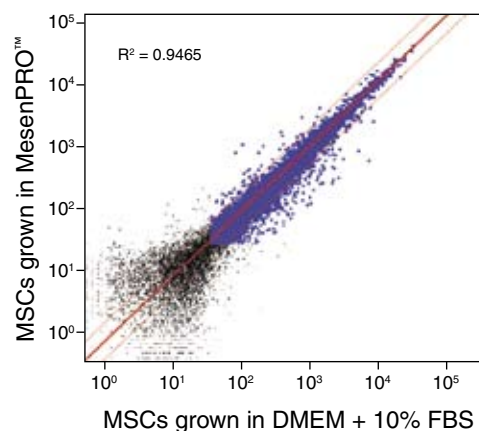


Figure 6—MesenPRO RS™ Medium maintains global mRNA expression of MSCs. MSCs were grown in media for 15 days (three subpassages, five days per subpassage, 3×10^3 cells/cm² per subpassage). Total RNA was isolated from MSCs grown in MesenPRO RS™ Medium, 2 mM L-glutamine, and 5 µg/ml gentamicin, or DMEM, 4 mM L-glutamine, 5 µg/ml gentamicin, and 10% FBS, and used for mRNA expression analysis using Illumina BeadArray™ technology. Data were analyzed using Illumina BeadStudio software (version 1.5.0.34); a pairwise comparison was made of the two samples and represented as a scatter plot. Data points with greater than 0.99 detection units are in blue, with points lying outside the red lines representing genes with greater than 2-fold differences. The R² correlation of 0.94, a value close to 1, suggests similar gene expression patterns between the MSCs grown in the two media.

Each MesenPRO RS™ Medium Kit includes MesenPRO RS™ Basal Medium and MesenPRO RS™ Growth Supplement (Figure 7). To learn more, ask your local Invitrogen Account Manager or visit www.invitrogen.com/stemcells today.



Figure 7—A complete MesenPRO RS™ Medium Kit

Get consistent results in your MSC culture every time. Use MesenPRO RS™ Medium.

Ordering Information

Product	Quantity	Cat. no.
MesenPRO RS™ Medium	1 kit	12746-012
MesenPRO RS™ Medium is shipped in two parts with separate storage requirements and catalog numbers: MesenPRO RS™ Basal Medium (500 ml, Cat. no. 12747-010) and MesenPRO RS™ Growth Supplement (10 ml, Cat. no. 12748-018). These components are not sold individually. For additional information and to place your order, please visit www.invitrogen.com/stemcell and www.invitrogen.com .		



www.invitrogen.com