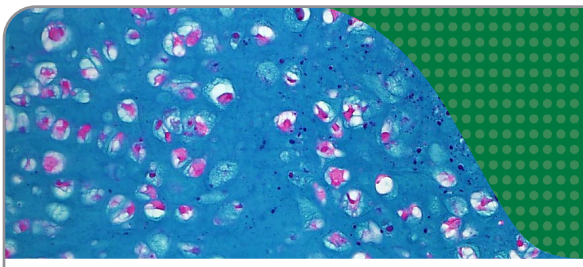

# MesenCult™-ACF Chondrogenic Differentiation Medium

## New Animal Component-Free, MSC Chondrogenic Differentiation

Mesenchymal stem cells (MSCs) are currently being investigated in tissue engineering and cell therapy research for cartilage repair, and a large effort focusing on understanding the mechanisms involved in MSC chondrogenic differentiation is underway.

In order to obtain sufficient cell numbers for chondrogenic differentiation in vitro, human MSCs are routinely isolated from bone marrow (BM) or adipose tissue and culture-expanded before differentiation to the chondrogenic lineage is performed. Traditionally, serum-based media are used to isolate, expand and differentiate human MSCs. The use of serum, however, is highly undesirable as there is inconsistent lot-to-lot performance, greater variability between experimental results and, in therapeutic applications, there are concerns about immune rejection of transplanted cells and disease transmission.

To overcome the issues surrounding the use of serum-based media, animal component-free **MesenCult™-ACF Chondrogenic Differentiation Medium** was developed for the efficient and robust differentiation of MSCs to the chondrogenic lineage. Using this medium, chondrogenic differentiation can be obtained with as few as 3 x $10^5$ human MSCs or in as little as 14 days in 3D pellet culture (Figure 1) with fewer hypertrophic chondrocytes present compared to competitor media (Figure 2). In addition, MesenCult™-ACF Chondrogenic Differentiation Medium induces stronger and more sustained chondrogenic transcript expression (Figure 3).

MesenCult™-ACF Chondrogenic Differentiation Medium has been optimized to be used in conjunction with other MesenCult™ animal component-free products, including MesenCult™-ACF Medium and MesenCult™-ACF Freezing Medium. Together, the derivation, expansion, cryopreservation and differentiation of MSCs to the chondrogenic lineage under defined, serum-free and animal component-free conditions is possible and is an important advancement for both basic and translational research.

### Advantages

- Defined, animal component-free (ACF) formulation
- Robust chondrogenic differentiation with as few as 3 x $10^5$ MSCs per 3D pellet and as early as day 14
- Strong expression of chondrogenic transcripts – Sox9, Acan, Col2a and Col10a; low expression of hypertrophic transcript Mmp13
- Completes optimized ACF workflow for MSC isolation, expansion, cryopreservation and chondrogenic differentiation when used with other MesenCult™-ACF media
- Compatible with MSCs previously expanded in MesenCult™ (Human), MesenCult™-XF or MesenCult™-ACF

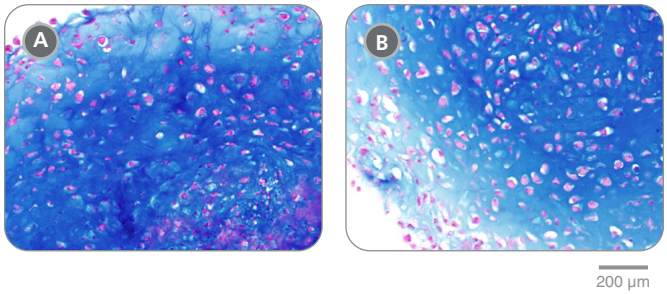


**Figure 1.** MesenCult™-ACF Chondrogenic Differentiation Medium Induces Robust Chondrogenic Differentiation of Human MSCs With As Few As 3 x $10^5$ Cells and As Early As Day 14

Human BM-derived MSCs were cultured in MesenCult™-ACF Medium then differentiated to the chondrogenic lineage using MesenCult™-ACF Chondrogenic Differentiation Medium. Robust chondrogenic differentiation was observed (A) starting with as few as 3 x $10^5$ MSCs, or (B) when differentiating for just 14 days starting with 5 x $10^5$ MSCs.

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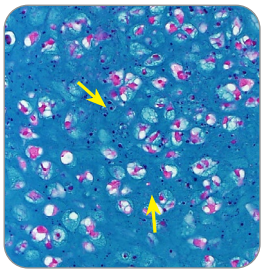
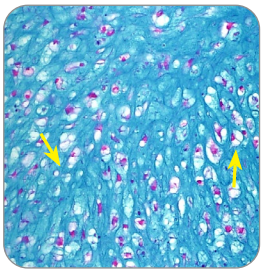
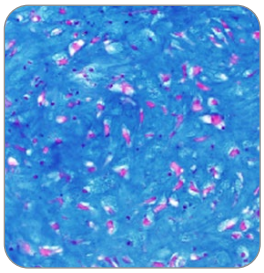
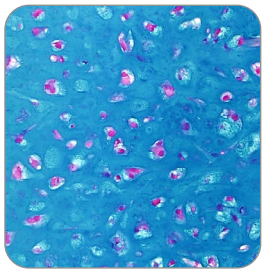
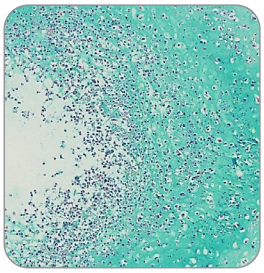
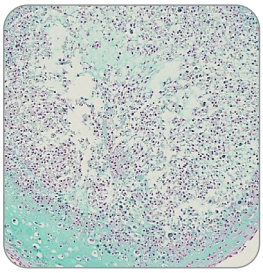
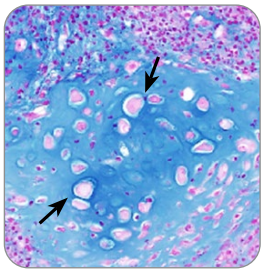
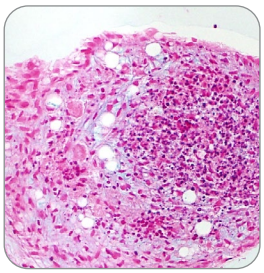
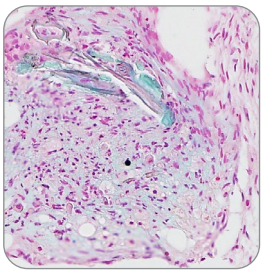
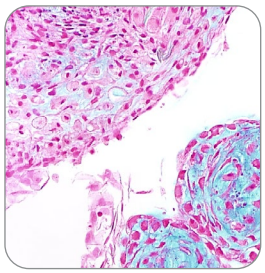
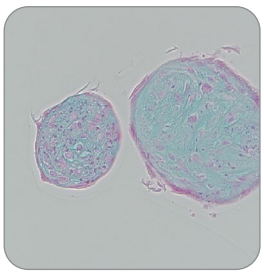
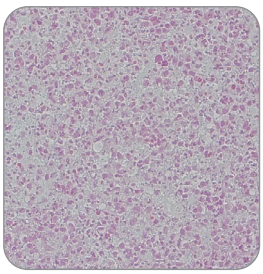
| CHONDROGENIC DIFFERENTIATION MEDIUM | EXPANSION MEDIUM | | | |
|---|---|---|---|---|
| | MesenCult™-ACF | Serum-Based | Competitor 1 (Ex1) | Competitor 2 (Ex2) |
| MesenCult™-ACF Chondrogenic Differentiation Medium |  |  |  |  |
| Competitor 1 (Ch1) Chondrogenic Differentiation Medium |  |  |  | Not performed |
| Competitor 2 (Ch2) Chondrogenic Differentiation Medium |  |  | Not performed |  |
| Competitor 3 (Ch3) Chondrogenic Differentiation Medium |  |  | Not performed | Not performed |

100 µm

**Figure 2.** Chondrogenic Differentiation of Human MSCs is More Robust with Fewer Hypertrophic Chondrocytes Using MesenCult™-ACF Chondrogenic Differentiation Medium Compared to Competitor Media

Human BM-derived MSCs culture-expanded for up to two passages in MesenCult™-ACF Medium, serum-based medium, or one of two commercially available media (Competitor 1 (Ex1) and Competitor 2 (Ex2)), were then differentiated to the chondrogenic lineage starting with 5 x $10^5$ MSCs and either using MesenCult™-ACF Chondrogenic Differentiation Medium or one of several commercially available chondrogenic differentiation media (Ch1, Ch2 or Ch3) for 21 days. More robust and uniform chondrogenic differentiation was observed when the MSCs were differentiated in MesenCult™-ACF Chondrogenic Differentiation Medium compared to the other commercially available chondrogenic differentiation media (Ch1, Ch2 and Ch3), irrespective of the expansion medium used to culture the MSCs prior to differentiation. Cultures differentiated using MesenCult™-ACF Chondrogenic Differentiation Medium displayed an abundance of isogenous groups (yellow arrows), suggesting there is proliferation of differentiating chondrocyte progenitors. Few hypertrophic chondrocytes (black arrows) are seen in cultures differentiated with MesenCult™-ACF Chondrogenic Differentiation Medium, suggesting the maintenance of chondrogenic activity throughout the culturing period.

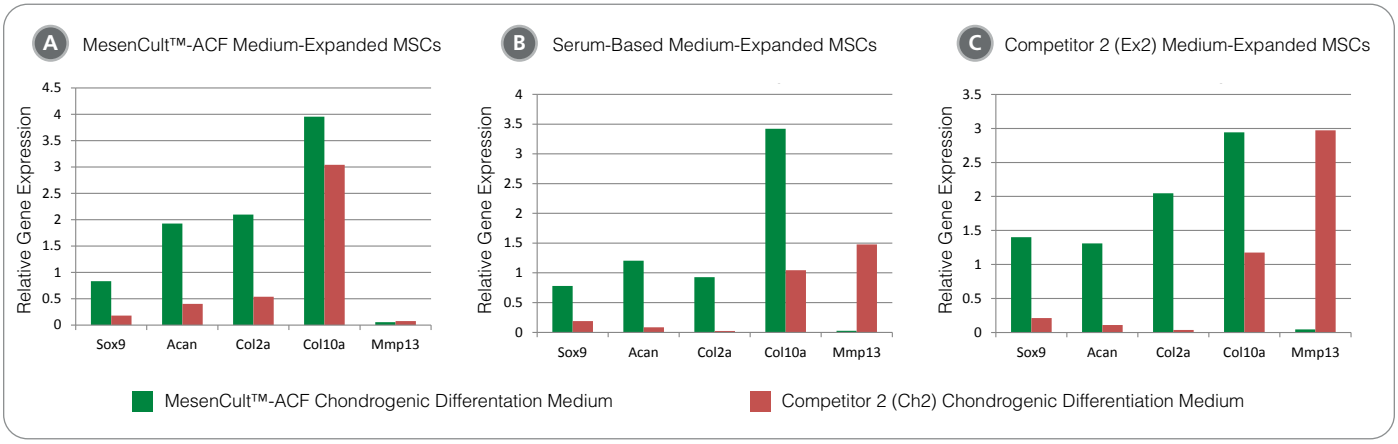



**Figure 3.** MesenCult™-ACF Chondrogenic Differentiation Medium Induces Stronger and More Sustained Chondrogenic Transcript Expression Compared to Competitor Media

Human BM-derived MSCs expanded in (A) MesenCult™-ACF Medium, (B) a serum-based medium or (C) Competitor 2 (Ex2) medium, were differentiated for 21 days with MesenCult™-ACF Chondrogenic Differentiation Medium and Competitor 2 (Ch2) chondrogenic differentiation medium. Regardless of the expansion medium initially used to culture the MSCs, differentiation using MesenCult™-ACF Chondrogenic Differentiation Medium led to a substantial up-regulation of the chondrogenic transcripts compared to Ch2. In addition, expression of the terminally-differentiated hypertrophic transcript Mmp13 was higher for Ch2 differentiated cultures compared to cultures differentiated with MesenCult™-ACF Chondrogenic Differentiation Medium.

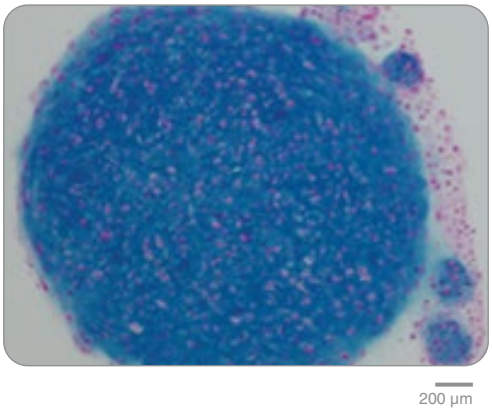



**Figure 4.** Mouse MSCs Cultured in MesenCult™-ACF Chondrogenic Differentiation Medium Differentiate to Chondrocytes

Mouse compact bone-derived MSCs were cultured using the MesenCult™ Proliferation Kit with MesenPure™ (Mouse; Catalog #05512) for 2 passages then differentiated by pellet culture with MesenCult™-ACF Chondrogenic Differentiation Medium for 21 days under normoxic (20% $O_2$) conditions. Strong chondrogenic differentiation is indicated by dark-blue staining of the cartilage extracellular matrix and an abundance of isogenous chondrocyte groups.

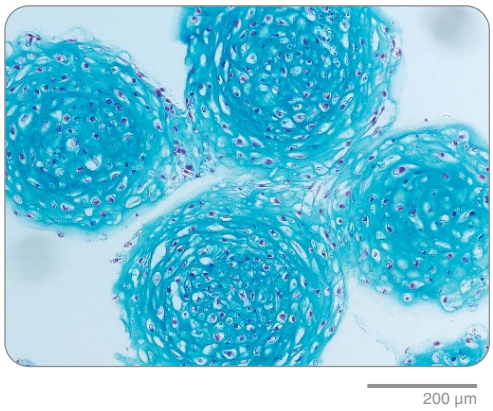



**Figure 5.** Human MSC Chondrogenic Differentiation with AggreWell™800 Plates

Using centrifugation, 1 x $10^6$ human MSCs were distributed evenly among 800 µm microwells in one well of an AggreWell™800 plate. Small aggregates of only ~3,300 cells per pellet were then differentiated to chondrocytes using MesenCult™-ACF Chondrogenic Differentiation Medium for 21 days under normoxic (20% $O_2$) conditions.

Aggregate (3D) pellet culture (Figure 6) is a standard method system for the chondrogenic differentiation of MSCs. MesenCult™-ACF Chondrogenic Differentiation Medium can be used in additional cell culture methods for MSC chondrogenic differentiation, including micromass culture, and with other cell types, such as mouse MSCs (Figure 4).

Chondrogenic differentiation with only a few thousand human MSCs per pellet can also easily be performed using AggreWell™800 plates (Figure 5). For more information on AggreWell™, visit **www.stemcell.com/aggrewell**.

# Animal Component-Free MSC Isolation, Expansion, Cryopreservation and Chondrogenic Differentiation

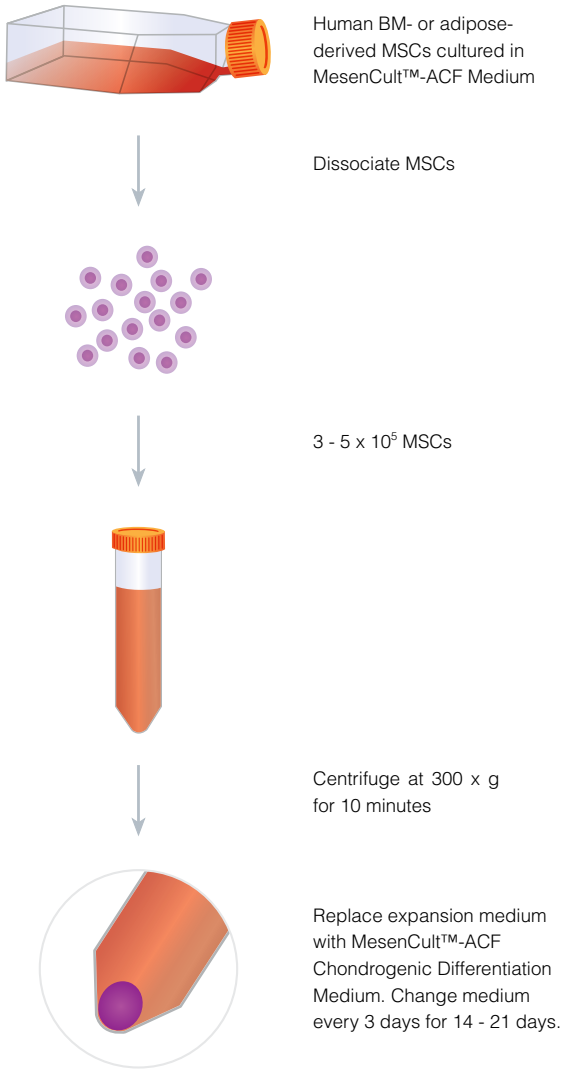



**Figure 6.** Procedure Overview: Human MSC Chondrogenic Differentiation Using MesenCult™-ACF Chondrogenic Differentiation Medium

Aggregate culture is a useful method for inducing chondrogenic differentiation of human BM- and adipose-derived MSCs in a three-dimensional in vitro culture environment. MSCs are efficiently differentiated to the chondrogenic lineage using MesenCult™-ACF Chondrogenic Differentiation Medium in 14 - 21 days with 3 - 5 x $10^5$ cells.

## Product Listing

| PRODUCT | QUANTITY | CATALOG # |
|---|---|---|
| MesenCult™-ACF Chondrogenic Differentiation Medium | 100 mL | 05455 New |
| MesenCult™-ACF Culture Kit* | 1 Kit | 05449 New |
| MesenCult™-ACF Medium | 500 mL | 05440 New |
| MesenCult™-ACF Dissociation Kit | 1 Kit | 05426 |
| MesenCult™-ACF Freezing Medium | 50 mL | 05490 |

*Contains MesenCult™-ACF Medium (#05440) and MesenCult™-ACF Attachment Substrate (Note: Substrate is only available as part of the MesenCult™-ACF Culture Kit).

For a complete list of related products for MSC research, including other differentiation media available from STEMCELL Technologies, please visit **www.stemcell.com/mesencult** or contact us at **techsupport@stemcell.com**.

STEMCELL Technologies' products are manufactured under a Quality Management System (QMS) certified to ISO 13485. Unless otherwise indicated, products are provided for research use only, not for human or animal therapeutic or diagnostic use.

Your regulatory authority will provide guidance on the requirements for ancillary materials for cell therapy applications. Depending on the requirements, STEMCELL may be able to assist you in meeting your regulatory and quality requirements.

STEMCELL Technologies stands behind the quality of our products. We welcome onsite audits of our manufacturing facilities to ensure that your quality requirements are met. If you have any questions or would like to discuss the potential use of a product for your application please contact us.