

# MesenCult™-ACF Chondrogenic Differentiation Kit



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Catalog #05455

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## Product Description

MesenCult™-ACF Chondrogenic Differentiation Medium is animal component-free (ACF) and specifically formulated for the in vitro differentiation of human mesenchymal stromal cells (MSCs; also known as mesenchymal stem cells) into chondrogenic lineage cells, including chondrocytes. This medium is suitable for the differentiation of human bone marrow (BM)-, adipose tissue (AT)- and synovium (S)-derived MSCs previously culture-expanded in serum-containing medium (e.g. MesenCult™ Proliferation Kit [Human; Catalog #05411], or MesenCult™-hPL Medium Kit [Catalog #05439]), or serum- and animal component-free MesenCult™-ACF Plus Medium (Catalog #05445). This medium is also suitable for the chondrogenic differentiation of human embryonic stem (ES) and induced pluripotent stem (iPS) cell-derived mesenchymal progenitor cells (MPCs) generated with STEMdiff™ Mesenchymal Progenitor Kit (Catalog #05240).

NOTE: Complete MesenCult™-ACF Chondrogenic Differentiation Medium must be supplemented with L-Glutamine (Catalog #07100).

## Product Information

The following components are sold as a complete kit (Catalog #05455) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
MesenCult™-ACF Chondrogenic Differentiation Basal Medium	05456	95 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.
MesenCult™-ACF 20X Chondrogenic Differentiation Supplement	05457	5 mL	Store at -20°C.	Stable until expiry date (EXP) on label.

## Preparation of Medium

Use sterile technique to prepare complete MesenCult™-ACF Chondrogenic Differentiation Medium (MesenCult™-ACF Chondrogenic Differentiation Basal Medium + MesenCult™-ACF 20X Chondrogenic Differentiation Supplement + L-Glutamine). The following example is for preparing 20 mL of complete medium. If preparing other volumes, adjust accordingly.

NOTE: For aliquoting and storing either the supplement or the complete medium, polypropylene tubes are strongly recommended (e.g. Falcon® Conical Tubes, 15 mL [Catalog #38009]).

1. Thaw MesenCult™-ACF 20X Chondrogenic Differentiation Supplement at room temperature (15 - 25°C). Alternatively, thaw supplement at 2 - 8°C overnight.

NOTE: Once thawed, use immediately or prepare 1 mL aliquots and store at -20°C. Do not exceed the expiry date as indicated on the label. After thawing the aliquots, use immediately. Do not re-freeze.

2. Add 1 mL of MesenCult™-ACF 20X Chondrogenic Differentiation Supplement to 19 mL of MesenCult™-ACF Chondrogenic Differentiation Basal Medium in a 50 mL polypropylene tube (e.g. Catalog #38010). Mix thoroughly.
3. Add 200 µL of 200 mM L-Glutamine (Catalog #07100) to reach a final concentration of 2 mM. Mix thoroughly.

NOTE: If not used immediately, store complete MesenCult™-ACF Chondrogenic Differentiation Medium at 2 - 8°C for up to 1 week. This medium does not contain antibiotics. If desired, add antibiotics and use medium within 1 week.

## Directions for Use

### 3D PELLET CULTURE SYSTEM FOR CHONDROGENIC DIFFERENTIATION OF MSCs AND hPSC-DERIVED MPCs

For differentiating to the chondrogenic lineage, it is recommended to use culture-expanded human MSCs between passages 1 - 4 and PSC-derived MPCs between passages 3 – 7. The following example is for preparing 4 pellets. If preparing other amounts, adjust accordingly. Warm complete MesenCult™-ACF Chondrogenic Differentiation Medium to room temperature (15 - 25°C) before use.

#### Day 0

1. Resuspend  $2.5 \times 10^5$  to  $5 \times 10^5$  MSCs or hPSC-derived MPCs in 2 mL of complete MesenCult™-ACF Chondrogenic Differentiation.
2. Add 0.5 mL of the cell suspension to each of 4 x 15 mL polypropylene tubes (e.g. Catalog #38009). Cap tightly and centrifuge at  $300 \times g$  for 5 - 10 minutes at room temperature.

NOTE: Ensure the tubes are polypropylene; do not use polystyrene or any other type material.

3. Very gently loosen the cap of each tube (while still keeping the cap on with a half twist) and place the tubes in a rack.
4. Incubate at 37°C and 5% CO<sub>2</sub> for 3 days.

#### Day 3

5. Gently add 0.5 mL of complete MesenCult™-ACF Chondrogenic Differentiation Medium to each tube, for a final volume of 1 mL. Incubate tubes in a rack at 37°C and 5% CO<sub>2</sub> for 3 days.

#### Day 6 - 21

6. On Day 6, and every 3 - 4 days afterward, carefully aspirate the medium without disturbing the pellet and replace with 0.5 mL of complete MesenCult™-ACF Chondrogenic Differentiation Medium. Incubate tubes in a rack at 37°C and 5% CO<sub>2</sub>.

NOTE: After each medium change, gently flick each tube to ensure the pellet is not completely attached to the tube.

NOTE: The pellets may significantly increase in size throughout the incubation period.

#### Day 21

7. The chondrogenic pellets have reached full differentiation and can be used for downstream applications, or for quantitative and qualitative characterization analysis. Histological sections of the pellet can be generated by fixing the pellets in 10% formalin at room temperature for 30 minutes, following subsequent standard paraffin embedding methods and staining 6 µm sections with Alcian Blue and Nuclear Fast Red.

## Notes and Tips

- Do not use less than 0.5 mL of complete medium per pellet culture.
- It is recommended to perform medium changes on a 3- to 4-day cycle. However, if the medium begins to turn yellow, switch to a 2-day cycle.

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