MesenCult™ Osteogenic Differentiation Kit (Human)

For the in vitro differentiation of human MSCs into osteoblasts

Catalog #05465 1 Kit

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Product Description

MesenCult™ Osteogenic Differentiation Kit (Human) is specifically formulated for the in vitro differentiation of human mesenchymal stem and progenitor cells (MSCs) into cells of the osteogenic lineage. This kit is suitable for the differentiation of human bone marrow (BM)- or adipose-derived MSCs previously culture-expanded in serum-containing medium (e.g. MesenCult™ Proliferation Kit [Human; Catalog #05411] or MesenCult™-hPL Medium [Human; Catalog #05439]) or serum- and animal component-free MesenCult™-ACF Plus Medium (Catalog #05445).

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

Product Information

The following components are sold as a complete kit (Catalog #05465) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
MesenCult™ Osteogenic Differentiation Basal Medium (Human)	05466	200 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
MesenCult™ Osteogenic Differentiation 5X Supplement (Human)	05467	50 mL	Store at -20°C.	Stable until expiry date (EXP) on label.

None of the above components contain antibiotics.

Preparation of Complete Osteogenic Differentiation Medium (Human)

Use sterile technique to prepare complete Osteogenic Differentiation Medium (Basal Medium + 5X Supplement + L-Glutamine). The following example is for preparing 50 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 the 5X Supplement at room temperature (15 - 25°C) or at 2 - 8°C overnight. Mix thoroughly.

   NOTE: Once thawed, use immediately or aliquot and store at -20°C. Do not exceed the shelf life of the supplement. After thawing the aliquoted supplement, use immediately. Do not re-freeze.
2. Add 10 mL of 5X Supplement to 40 mL of Basal Medium. Mix thoroughly.
3. Add 0.5 mL of L-Glutamine (200 mM; Catalog #07100) to reach a final concentration of 2 mM. Mix thoroughly.

NOTE: If not used immediately, store complete Osteogenic Differentiation Medium at 2 - 8°C for up to 1 week.

Directions for Use

Please read the entire protocol before proceeding.

For instructions on culturing human MSCs using the MesenCult™ media listed below, refer to the Product Information Sheets available at www.stemcell.com.

- MesenCult™ Medium (Human; Catalog #05411)
- MesenCult™-hPL Medium (Human; Catalog #05439)
- MesenCult™-ACF Plus Medium (Catalog #05445)

For differentiating to the osteogenic lineage, use culture-expanded human MSCs between passages 1 - 4.

The following protocol is for setting up differentiation assays using human BM- or adipose-derived MSCs in a 6-well plate. If using other cultureware, adjust volumes accordingly.

NOTE: Only use tissue culture-treated cultureware.

1. Plate cells in 2 mL of growth medium per well. Recommended cell plating densities are as follows:
   - MesenCult™ Medium (Catalog #05411): 8 - 16 x 10^3 cells/cm^2
   - MesenCult™-ACF Plus Medium or MesenCult™-hPL Medium (Human): 4 - 8 x 10^3 cells/cm^2

   NOTE: If using MesenCult™-ACF Plus Medium, ensure to coat cultureware as described in the Product Information Sheet (Document #10000003462).
2. Incubate at 37°C in 5% CO_2 with ≥ 95% humidity until cells are approximately 90 - 98% confluent. This takes approximately 1 - 5 days depending on the expansion medium used.
3. Aspirate medium and replace with 2 mL of complete Osteogenic Differentiation Medium per well. Incubate at 37°C in 5% CO_2 with ≥ 95% humidity.
4. Change medium every 3 - 4 days until bone matrix formation occurs (approximately 10 - 15 days).
5. Visualize osteogenic differentiation by staining with Alizarin Red S, alkaline phosphatase, or silver nitrate (von Kossa).

   NOTE: The level of osteogenic differentiation for MSCs may vary depending on cell source, donor, and previous culture conditions.