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 stromal cells (MSCs; also known as mesenchymal stem cells) into cells of the osteogenic lineage. This kit is suitable for the differentiation of human bone marrow (BM)- or adipose tissue (AT)-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). This kit is also suitable for the osteogenic 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™ 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.

Preparation of Medium

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). Alternatively, thaw supplement 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. This medium does not contain antibiotics.

Directions for Use

Please read the entire protocol before proceeding.

For instructions on culturing human MSCs or ES/iPS-derived MPCs using the MesenCult™ media listed below, or instructions on derivation and expansion of cells with MPC-like properties from human ES or iPS cells using STEMdiff™ Mesenchymal Progenitor Kit (Catalog #05240), refer to the following 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)
- MesenCult™-ACF Plus Culture Kit (Catalog #05448)

For differentiating to the osteogenic lineage, use culture-expanded human MSCs between passages 1 - 4 and culture-expanded PSC-derived MPCs between passages 4 - 14.

The following protocol is for setting up differentiation assays using human BM- or AT-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 until cells are approximately 80 - 90% 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.
4. Change medium every 3 - 4 days until bone matrix formation occurs. The culture time for inducing differentiation is dependent on the cell source. See Table 1 for typical ranges.

Table 1. Culture Times for Inducing Osteogenic Differentiation for Various MSC Sources

MSC SOURCE	CULTURE TIMES FOR DIFFERENTIATION (DAYS)
Bone Marrow	10 - 15
Adipose Tissue	10 - 15
PSCs	14 - 21

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.