MesenCult™ Osteogenic Stimulatory Kit (Human)

Medium for differentiation of human mesenchymal stem cells into osteogenic progenitor cells

Catalog #05404 1 Kit

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Product Description

MesenCult™ Osteogenic Stimulatory 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)-derived MSCs previously culture-expanded in serum-containing medium (e.g. MesenCult™ Proliferation Kit [Catalog #05411]). This kit can be used for the detection of human colony-forming unit–osteoblast (CFU-O) and can also support CFU-O assays using rat BM-derived MSCs.

This kit should not be used on cells previously cultured with MesenCult™-XF Medium (Catalog #05420). To differentiate MSCs previously cultured in MesenCult™-XF Medium into osteogenic progenitor cells, use MesenCult™ Osteogenic Stimulatory Kit (for MSCs cultured in MesenCult™-XF; Catalog #05434).

Product Information

Aliquot and store components upon receipt as described below.

PRODUCT NAME	CATALOG #	SIZE	STORAGE	SHELF LIFE	USAGE
MesenCult™ MSC Basal Medium (Human)	05401	450 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.	• Aliquot into 10 x 45 mL • Store at 2 - 8°C
MesenCult™ Osteogenic Stimulatory Supplement (Human)*†	05405	80 mL	Store at -20°C.	Stable until expiry date (EXP) on label.	• Use at 15% final volume • Aliquot into 10 x 8 mL • Store at -20°C
β-Glycerophosphate (1 M)*	05406	10 mL	Store at -20°C.	Stable until expiry date (EXP) on label.	• Use at 3.5 mM final concentration • Aliquot into 10 x 1 mL • Store at -20°C
Dexamethasone*‡	05407	1 mg	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.	• Use at 10^-8 M final concentration • Prepare a 0.1 mg/mL solution by dissolving 1 mg of powder in 10 mL of absolute ethanol • Prepare a 10^-4 M stock solution by adding 7.75 mL of MesenCult™ MSC Basal Medium (Human) to 5 mL of 0.1 mg/mL Dexamethasone • Aliquot into 25 x 500 µL • Store at 2 - 8°C
Ascorbic Acid	07157	100 mg	Store at 15 - 25°C.	Not applicable.	• Use at 50 µg/mL final concentration • To prepare a 10 mg/mL stock solution, dissolve powder in 10 mL of MesenCult™ MSC Basal Medium (Human) • Aliquot into 10 x 1 mL • Store at 2 - 8°C

*This component is sold as part of MesenCult™ Osteogenic Stimulatory Kit (Human; Catalog #05404) and is not available for individual sale.
†MesenCult™ Osteogenic Stimulatory Supplement (Human) contains proprietary supplements that have been pretested and selected for their ability to optimally differentiate human MSCs into cells of the osteogenic lineage.
‡Please refer to the Safety Data Sheet (SDS) for hazard information.

None of the above components contain antibiotics.

Preparation of Complete MesenCult™ Osteogenic Stimulatory Medium (Human)

NOTE: Complete MesenCult™ Osteogenic Stimulatory Medium should be prepared in volumes that can be used within 1 week. To facilitate this, reagents should be aliquoted and stored upon arrival as described in the Product Information section.

Use sterile techniques to prepare complete MesenCult™ Osteogenic Stimulatory Medium (Basal Medium + Osteogenic Stimulatory Supplement + Dexamethasone + Ascorbic Acid + β-Glycerophosphate). The following example is for preparing 50 mL of complete medium. If preparing other volumes, adjust accordingly.

NOTE: **β-Glycerophosphate is not added to the medium at the start of the assay** (see Directions for Use, step 3). Typically, β-Glycerophosphate is only added after evidence of cell multilayering is observed by phase contrast microscopy.

1. Add 42.5 mL of MesenCult™ MSC Basal Medium (Human) to a 50 mL tube (e.g. Catalog #38010).
2. Add the following components:
   - 7.5 mL of MesenCult™ Osteogenic Stimulatory Supplement (Human)
   - 5 µL of Dexamethasone (10^-4 M stock solution)
   - 250 µL of Ascorbic Acid (10 mg/mL stock solution)

   After multilayering is observed:
   - 175 µL of β-Glycerophosphate (1 M)

Directions for Use

Please read the entire protocol before proceeding.

For instructions on culturing human MSCs using complete MesenCult™ Medium (MesenCult™ Proliferation Kit [Human]; Catalog #05411) refer to the Product Information Sheet (Document #29562) available at www.stemcell.com or contact us to request a copy.

For differentiating to the osteogenic lineage, it is recommended to use culture-expanded human MSCs between passage 1 - 4.
For differentiating rat MSCs to the osteogenic lineage, please contact us at techsupport@stemcell.com.

The following protocol is for setting up differentiation assays using human BM-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 (e.g. complete MesenCult™ Medium) per well. The recommended plating density with MesenCult™ Medium (Catalog #05411) is 2.5 - 6 x 10^3 cells/cm^2.
2. Incubate at 37°C until cells are approximately 70 - 80% confluent. This takes approximately 1 - 5 days.
3. Aspirate medium and replace with 2 mL of complete MesenCult™ Osteogenic Stimulatory Medium (**without** β-Glycerophosphate) per well.
4. Examine cells using phase contrast microscopy and monitor for the presence of cell multilayering (i.e. layering of cells on top of each other, forming a matrix, as opposed to growing in a planar manner). This takes approximately 2 - 7 days.

   NOTE: Multilayering is indicative of the beginning of bone generation. If cell multilayering has been observed, supplement complete MesenCult™ Osteogenic Stimulatory Medium with β-Glycerophosphate (see Preparation of Complete MesenCult™ Osteogenic Stimulatory Medium [Human]).
5. Once multilayering has been observed, aspirate medium and replace with 2 mL of complete MesenCult™ Osteogenic Stimulatory Medium (**with** β-Glycerophosphate) per well.
6. Incubate cells at 37°C and change medium every 3 days using 2 mL of complete MesenCult™ Osteogenic Stimulatory Medium (**with** β-Glycerophosphate) per well. The differentiation assay takes approximately 2 - 4 weeks. During this time, calcium deposition should be easily observed under low magnification.
7. Osteogenic differentiation may be visualized by Alizarin Red S, alkaline phosphatase, or silver nitrate (von Kossa) staining.

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