MesenCult™ Osteogenic Stimulatory Kit (Mouse)

Complete Medium for Differentiating Mouse Mesenchymal Stem Cells and Embryonic Fibroblasts into Osteoblasts

Catalog #05504 250 mL

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Product Description

The MesenCult™ Osteogenic Stimulatory Kit (Mouse) is specifically formulated for the in vitro differentiation of mouse mesenchymal stem and progenitor cells (MSCs) and mouse embryonic fibroblasts (MEFs) into cells of the osteogenic lineage, including osteoblasts.

This kit is suitable for the differentiation of mouse bone marrow (BM)-derived MSCs, compact bone (CB)-derived MSCs, and adipose-derived MSCs and MEFs. It contains proprietary supplements that have been pretested and selected for their ability to optimally differentiate mouse MSCs into cells of the osteogenic lineage.

Product Information

The following components are sold as a complete kit (Catalog #05504) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
MesenCult™ MSC Basal Medium (Mouse)	05505	200 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.
MesenCult™ Osteogenic Stimulatory Supplement (Mouse)	05506	50 mL	Store at -20°C.	Stable until expiry date (EXP) on label.

None of the above components contain antibiotics.

Preparation of Complete MesenCult™ Osteogenic Medium (Mouse)

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

1. Thaw Osteogenic Stimulatory Supplement (Mouse) 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 until expiry date as indicated on the label. After thawing the aliquoted supplement, use immediately. Do not re-freeze.

2. Add 10 mL of Stimulatory Supplement to 40 mL of Basal Medium. Mix thoroughly.

   NOTE: If not used immediately, store complete MesenCult™ osteogenic medium at 2 - 8°C for up to 1 month. Do not exceed the shelf life of the individual components.

   NOTE: MesenCult™ MSC Basal Medium (Mouse) stored for more than 2 months following the date of manufacture, as indicated on the label, should be supplemented with additional L-glutamine. Add 1 mL of 200 mM L-Glutamine (Catalog #07100) to 99 mL of medium to achieve a final concentration of 2 mM.

Directions for Use

Please read the entire protocol before proceeding.

For instructions on culturing mouse MSCs and MEFs in complete MesenCult™ medium using the MesenCult™ Proliferation Kit with MesenPure™ (Mouse; Catalog #05512) or without MesenPure™ (MesenCult™ MSC Basal Medium [Catalog #05501] + MesenCult™ Stimulatory Supplements [Catalog #05502]), refer to the Product Information Sheet (PIS; Document #29566) available on our website at www.stemcell.com or contact us to request a copy.

NOTE: It is important that the starting MSC population has a reduced number of unwanted hematopoietic cells prior to osteogenic differentiation. Enriched cultures of MSCs may be obtained using the MesenCult™ Proliferation Kit with MesenPure™ (Mouse), the EasySep™ Mouse Mesenchymal Stem/Progenitor Cell Enrichment Kit (Catalog #19771), or enriching culture-expanded MSCs from other tissue sources.

For optimal results, culture MSCs and MEFs under hypoxic conditions consisting of 5% O_2 and 5 - 10% CO_2, at 37°C in a humidified cell culture incubator or use the Hypoxia Incubator Chamber (Catalog #27310). For instructions on how to use the Hypoxia Incubator Chamber refer to the PIS (Document #29829) available on our website at www.stemcell.com or contact us to request a copy.

For differentiating cells into the osteogenic lineage, it is recommended to use culture-expanded mouse MSCs and MEFs expanded between passage 1 - 3.

1. Plate cells in appropriate proliferation medium (e.g. complete MesenCult™ medium with or without MesenPure™). Refer to Table 1 for recommended cell plating densities.

   NOTE: The addition of MesenPure™ to complete MesenCult™ proliferation medium is strongly recommended to maximize enrichment of MSC and MEF cultures.

Table 1: Recommended Cell Plating Densities in Complete MesenCult™ Medium With and Without MesenPure™

CELL TYPE	CELLS PER cm^2 IN COMPLETE MESENCULT™ MEDIUM	
	With MesenPure™ (Catalog #05512)	Without MesenPure™ (Catalog #05501 + 05502)
BM-derived MSCs	4 - 6 x 10^4	10 - 20 x 10^4
CB-derived MSCs	4 - 6 x 10^4	10 - 20 x 10^4
Adipose-derived MSCs	3 - 6 x 10^4	
EasySep™ enriched CB-derived MSCs	4 - 6 x 10^4	
MEFs	3 - 6 x 10^4	

2. Incubate cells at 37°C under hypoxic conditions until they are approximately 80 - 90% confluent. This takes approximately 1 - 3 days.
3. Aspirate medium and replace with complete MesenCult™ osteogenic medium.
4. Incubate cells at 37°C in hypoxic conditions and change medium every 3 days using complete MesenCult™ osteogenic medium until bone matrix formation is observed. This takes approximately 14 - 21 days.
5. Osteogenic differentiation may be detected by Alizarin Red S or silver nitrate (von Kossa) staining, by qPCR analysis of bone-specific transcripts, or by another appropriate assay.