MesenCult™ Osteogenic Stimulatory Kit (Mouse)

Medium for differentiating mouse mesenchymal stem cells and embryonic fibroblasts into osteoblasts

Catalog #05504 250 mL

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

MesenCult™ Osteogenic Stimulatory Kit (Mouse) is specifically formulated for the in vitro differentiation of mouse mesenchymal stem and progenitor cells (MSCs) from compact bone (CB) and bone marrow (BM), adipose tissue-derived MSCs (ADSCs), or mouse embryonic fibroblasts (MEFs) into osteoblasts. This kit induces strong osteogenesis of mouse MSCs and MEFs, as evidenced by qPCR analysis of key transcripts involved in bone differentiation and maturation, and alkaline phosphatase activity and silver nitrate (von Kossa method) staining. This kit is recommended for characterizing MSCs and MEFs and studying bone development.

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

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 + L-Glutamine). 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 overnight at 2 - 8°C. 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.
3. Add 0.5 mL of L-Glutamine (Catalog #07100) to give a final concentration of 2 mM. Mix thoroughly.

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

Directions for Use

Please read the entire protocol before proceeding.

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 MesenCult™ Expansion Kit (Mouse; Catalog #05513), 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 Product Information Sheet (Document #29829) available 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™ Expansion Medium with or without MesenPure™). Refer to Table 1 for recommended cell plating densities.

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

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

CELL TYPE	CELL PLATING DENSITY (cells/cm^2 in complete MesenCult™ Expansion Medium [Catalog #05513])	
	With MesenPure™	Without MesenPure™
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
ADSCs	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 > 90% confluent. This takes approximately 1 - 3 days.
3. Aspirate medium and replace with complete MesenCult™ Osteogenic Medium.

   NOTE: Do not add MesenPure™ to 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 10 - 12 days for BM- and CB-derived MSCs, or 14 - 21 days for ADSCs and MEFs.
5. Osteogenic differentiation may be detected by alkaline phosphatase activity or silver nitrate (von Kossa) staining, by qPCR analysis of bone-specific transcripts, or by another appropriate assay.