MesenCult™ Adipogenic Stimulatory Supplement (Mouse)

Supplement for differentiating mouse mesenchymal stem cells into adipocytes

Catalog #05503 100 mL

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Product Description

MesenCult™ Adipogenic Stimulatory Supplement (Mouse), when used in conjunction with Alpha MEM without Nucleosides (Catalog #36453), comprises a complete medium specifically formulated for the in vitro differentiation of mouse mesenchymal stem and progenitor cells (MSCs) and mouse embryonic fibroblasts (MEFs) into cells of the adipogenic lineage. MesenCult™ Adipogenic Stimulatory Supplement (Mouse) contains proprietary supplements that have been pretested and selected for their ability to optimally differentiate mouse MSCs into cells of the adipogenic lineage. This product does not contain antibiotics.

Complete MesenCult™ Adipogenic Medium (Mouse) is suitable for the differentiation of mouse bone marrow (BM)-derived MSCs, compact bone (CB)-derived MSCs, and adipose-derived MSCs and MEFs.

Properties

Storage: Store at -20°C.

Shelf Life: Stable until expiry date (EXP) on label.

Preparation of Complete MesenCult™ Adipogenic Medium (Mouse)

Use sterile techniques to prepare complete MesenCult™ Adipogenic Medium (Alpha MEM without Nucleosides + MesenCult™ Adipogenic Stimulatory Supplement). The following example is for preparing 500 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw MesenCult™ Adipogenic Stimulatory 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 until expiry date as indicated on the label. After thawing the aliquots, use immediately. Do not re-freeze.
2. Add 100 mL of MesenCult™ Adipogenic Stimulatory Supplement to 400 mL of Alpha MEM without Nucleosides. Mix thoroughly.

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

   NOTE: Alpha MEM without Nucleosides 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™ Expansion Medium (Mouse; Catalog #05513), refer to the Product Information Sheet (PIS; Document #DX21764) available 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 adipogenic differentiation. Enriched MSC cultures 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 using the Hypoxia Incubator Chamber, refer to the PIS (Document #29829) available at www.stemcell.com or contact us to request a copy.

For differentiating cells into the adipogenic lineage, use culture-expanded mouse MSCs and MEFs expanded between passages 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
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™ Adipogenic Medium.

   NOTE: Do not add MesenPure™ to complete MesenCult™ Adipogenic Medium.
4. Incubate cells at 37°C in hypoxic conditions and change medium every 3 days using complete MesenCult™ Adipogenic Medium until lipid vacuoles are observed. This takes approximately 14 - 21 days.
5. Adipogenic differentiation may be detected by Oil Red O staining, by qPCR analysis of adipogenic-specific transcripts, or by another appropriate assay.