MesenCult™ Adipogenic Differentiation Kit (Mouse)

For the in vitro differentiation of mouse MSCs, ADSCs, and MEFs into adipocytes

Catalog #05507 222 mL

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Product Description

MesenCult™ Adipogenic Differentiation Kit (Mouse) is specifically formulated for the in vitro differentiation of mouse mesenchymal stem and progenitor cells (MSCs), adipose tissue-derived MSCs (ADSCs), and mouse embryonic fibroblasts (MEFs) into cells of the adipogenic lineage.

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

Product Information

The following components are sold as a kit (Catalog #05507) 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™ Adipogenic Differentiation 10X Supplement (Mouse)	05509	22 mL	Store at -20°C.	Stable until expiry date (EXP) on label.

None of the above components contain antibiotics.

Preparation of MesenCult™ Adipogenic Differentiation Medium (Mouse)

Use sterile techniques to prepare complete MesenCult™ Adipogenic Differentiation Medium (Basal Medium + 10X Supplement + L-Glutamine). The following example is for preparing 50 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw 10X 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. Do not exceed the shelf life of the supplement. After thawing the aliquoted supplements, use immediately. Do not re-freeze.
2. Add 5 mL of 10X Supplement to 45 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 MesenCult™ Adipogenic Differentiation 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.

For instructions on culturing mouse MSCs, ADSCs, and MEFs in complete MesenCult™ Expansion Medium using MesenCult™ Expansion Kit (Mouse; Catalog #05513) with or without MesenPure™, refer to the Product Information Sheet (PIS) Document #DX21764, available at www.stemcell.com or contact us to request a copy.

It is important that the starting MSC population has a reduced number of unwanted hematopoietic cells prior to adipogenic 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 by enriching culture-expanded MSCs from other tissue sources.

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

For optimal differentiation of cells into the adipogenic lineage, use culture-expanded mouse MSCs, ADSCs, or MEFs between passage 1 - 3.

1. Plate cells in an 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, ADSC, and MEF cultures.

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

CELL TYPE	PLATING DENSITY (cells per cm^2)	
	With MesenPure™ (Catalog #05513)	Without MesenPure™ (Catalog #05514 + 05515)
BM-derived MSCs	1 - 1.5 x 10^4	2 - 3 x 10^4
CB-derived MSCs	1 - 1.5 x 10^4	
ADSCs	1 - 1.5 x 10^4	
EasySep™-enriched CB-derived MSCs	4 - 6 x 10^4	
MEFs	1 - 1.5 x 10^4	

2. Incubate at 37°C in hypoxic conditions (5% O_2 and 5% CO_2) until cells are approximately > 90% confluent. This takes approximately 1 - 3 days.
3. Aspirate medium and replace with MesenCult™ Adipogenic Differentiation Medium.
4. Incubate cells at 37°C in hypoxic conditions. Change medium every 3 - 4 days using MesenCult™ Adipogenic Differentiation Medium until lipid droplets are observed. This takes approximately 5 - 7 days for BM-derived MSCs, or 10 - 14 days for CB-derived MSCs, ADSCs, and MEFs.

   NOTE: Adipogenic differentiation may be detected by Oil Red O staining, by qPCR analysis of adipogenic-specific transcripts, or by another appropriate assay.