MesenCult™ Adipogenic Differentiation Kit (Human)

Medium for differentiation of human MSCs into adipocytes

Catalog #05412 1 Kit

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Product Description

MesenCult™ Adipogenic Differentiation Medium (Human) is specifically formulated for the in vitro differentiation of human mesenchymal stromal cells (also known as mesenchymal stem cells or MSCs) into adipogenic lineage cells. This kit is suitable for the differentiation of MSCs derived from human bone marrow (BM), adipose tissue, umbilical cord, or pluripotent stem cells (PSCs) that have been previously culture-expanded in serum- and animal component-free medium (e.g. MesenCult™-ACF Plus Medium [Catalog #05445]), serum-containing medium (e.g. MesenCult™ Proliferation Kit [Catalog #05411]), or platelet lysate medium (e.g. MesenCult™-hPL Medium [Catalog #05439]).

Product Information

The following components are sold as a complete kit (Catalog #05412) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
MesenCult™ MSC Basal Medium (Human)	05413	225 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.
MesenCult™ 10X Adipogenic Differentiation Supplement (Human)	05414	25 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
MesenCult™ 500X Adipogenic Differentiation Supplement (Human)*	05415	0.5 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.

*Please refer to the Safety Data Sheet (SDS) for hazard information. This product contains components dissolved in dimethyl sulfoxide (DMSO). DMSO is a strong solvent and skin penetrant, and can transport many substances through the skin. DMSO can also penetrate some protective glove materials including latex and silicone. Extra caution should be utilized when handling this product.

None of the above components contain antibiotics.

Preparation of Complete MesenCult™ Adipogenic Differentiation Medium (Human)

Use sterile technique to prepare complete MesenCult™ Adipogenic Differentiation Medium (Basal Medium + 10X Supplement + 500X Supplement + L-Glutamine). The following example is for preparing 250 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw MesenCult™ 10X Adipogenic Differentiation Supplement (Human) and MesenCult™ 500X Adipogenic Differentiation Supplement (Human) at room temperature (15 - 25°C) or at 2 - 8°C overnight. Thoroughly mix the MesenCult™ 10X Adipogenic Differentiation Supplement (Human).

   NOTE: Once thawed, use immediately or aliquot and store supplements at -20°C. Do not exceed the shelf life of the supplements. After thawing the aliquoted supplements, use immediately. Do not re-freeze.
2. Add 25 mL of MesenCult™ 10X Adipogenic Differentiation Supplement (Human) and 0.5 mL of MesenCult™ 500X Adipogenic Differentiation Supplement (Human) to 222.5 mL of MesenCult™ MSC Basal Medium (Human). Mix thoroughly.
3. Add 2.5 mL of 200 mM L-Glutamine (Catalog #07100) to give a final concentration of 2 mM. Mix thoroughly.

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

Directions for Use

Please read the entire protocol before proceeding.

For instructions on culturing human MSCs using serum- and animal component-free MesenCult™-ACF Plus Medium or MesenCult™ Medium (MesenCult™ Proliferation Kit [Human]), refer to the Product Information Sheets, available at www.stemcell.com or contact us to request a copy.

For differentiating to the adipogenic lineage, it is recommended to use culture-expanded human MSCs between passages 1 - 4.

Adipogenic differentiation is a feature of more adult cell types, and PSC-derived MPCs tend to become more mature with extended passaging. Therefore, PSC-derived MPCs are in optimal conditions for adipogenic differentiation after P10.

The following protocol is for setting up differentiation assays using BM-, adipose-, umbilical-, or PSC-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 per well. See Table 1 for recommended cell plating densities.

   NOTE: If using MesenCult™-ACF Plus Medium, ensure that cells are plated on wells previously coated with Animal Component-Free Cell Attachment Substrate (included in MesenCult™-ACF Plus Culture Kit [Catalog #05448]).

Table 1. Recommended Cell Plating Densities

GROWTH MEDIUM	PLATING DENSITY (cells/cm^2)
MesenCult™-ACF Plus Medium (Catalog #05445)	1.5 - 3 x 10^3
MesenCult™ Proliferation Kit (Human; Catalog #05411)	2.5 - 6 x 10^3

2. Incubate cells at 37°C until they are approximately 90 - 100% confluent. This takes approximately 1 - 7 days.
3. Aspirate medium and replace with 2 mL of complete MesenCult™ Adipogenic Differentiation Medium per well.
4. Incubate cells at 37°C and change medium every 3 - 4 days using 2 mL of complete MesenCult™ Adipogenic Differentiation Medium per well. The culture time for inducing differentiation is dependent on cell source. See Table 2 for typical ranges. During this time, lipid vacuoles should be easily observed under low magnification.

Table 2. Culture Times for Inducing Adipogenic Differentiation for Various MSC Sources

MSC SOURCE	CULTURE TIME FOR DIFFERENTIATION (days)
Bone marrow	10 -14
Adipose tissue	10 -14
Umbilical cord	25 - 35
PSCs	21+

5. Adipogenic differentiation may be visualized by Oil Red O staining.

   NOTE: Level of adipogenic differentiation for MSCs may vary depending on cell source, donor, and previous culture conditions.

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