PRODUCT DESCRIPTION

Adipogenic Stimulatory Supplement (Mouse), when used in conjunction with MesenCult® MSC Basal Medium (Mouse; Catalog #05501), supports the *in vitro* differentiation of mouse mesenchymal stem cells (MSCs) into adipocytes.

COMPONENTS

Adipogenic Stimulatory Supplement (Mouse; Catalog #05503) contains serum and proprietary supplements which have been pretested and selected for their ability to optimally differentiate mouse mesenchymal stem cells into adipogenic cells.

This product is a biological reagent, and as such cannot be completely characterized or quantified.

This product has been aseptically manufactured using tightly controlled processes and is sterility tested.

STABILITY AND STORAGE

Product stable at -20°C until expiry date as indicated on the label. Long-term storage at 2 - 8°C is not recommended.

Adipogenic Stimulatory Supplement (Mouse) can be thawed, aliquoted into smaller volumes and refrozen to facilitate the preparation of small volumes of Complete MesenCult® Adipogenic Medium (Mouse). Do not freeze-thaw more than twice.

Once Adipogenic Stimulatory Supplement (Mouse; Catalog #05503) has been added to MesenCult® MSC Basal Medium (Mouse; Catalog #05501), the Complete MesenCult® Adipogenic Medium (Mouse) can be stored for up to 1 month at 2 - 8°C.

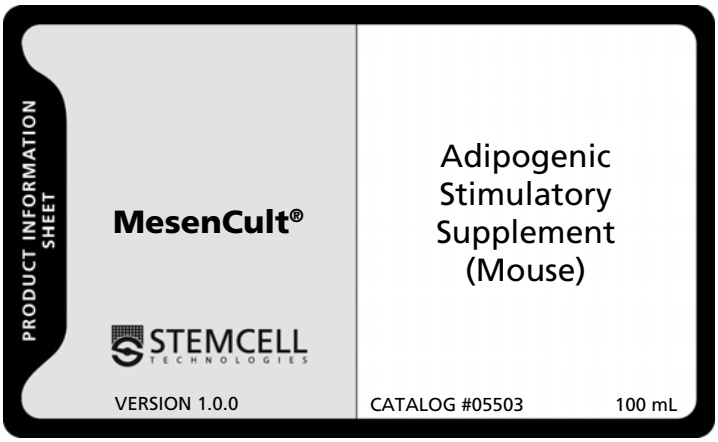
RELATED PRODUCTS

PRODUCT	CATALOG #
MesenCult® MSC Basal Medium (Mouse)	05501
MesenCult® Proliferation Kit (Mouse)	05511
MesenCult® MSC Proliferation Supplements (Mouse)	05502
EasySep® Mouse Mesenchymal Progenitor Enrichment Kit for Compact Bone	19771
L-Glutamine	07100

DIRECTIONS FOR USE

For additional instructions on isolating and culturing mouse mesenchymal stem cells, please refer to the Technical Manual for Enumeration and Expansion of Mouse Mesenchymal Stem Cells using MesenCult® (Manual Catalog #28374) available on our website at www.stemcell.com/technical/manuals.aspx or contact us to request a copy.

Antibiotics are not present in Adipogenic Stimulatory Supplement (Mouse; Catalog #05503), MesenCult® MSC Basal Medium (Mouse; Catalog #05501) or MesenCult® MSC Proliferation Supplements (Mouse; Catalog #05502) and can be added if desired when preparing the complete media.



1.0 Preparation of 500 mL Complete MesenCult® Adipogenic Medium (Mouse):

1. Thaw the Adipogenic Stimulatory Supplement (Mouse; Catalog #05503) overnight at 2 - 8°C or for several hours at room temperature (15 - 25°C) prior to usage. The supplement can be aliquoted into smaller volumes and stored at -20°C.
2. Add the entire 100 mL Adipogenic Stimulatory Supplement (Mouse) to 400 mL MesenCult® MSC Basal Medium (Mouse; Catalog #05501) and mix thoroughly. This is now referred to as Complete MesenCult® Adipogenic Medium (Mouse). If less than 500 mL will be required in a 1 month period, smaller volumes can be prepared.

   e.g. Prepare Complete MesenCult® Adipogenic Medium (Mouse) by diluting Adipogenic Stimulatory Supplement (Mouse) 1/5 with MesenCult® MSC Basal Medium (Mouse). For example, prepare 100 mL Complete MesenCult® Adipogenic Medium (Mouse) by adding 20 mL Adipogenic Stimulatory Supplement (Mouse) to 80 mL MesenCult® MSC Basal Medium (Mouse).

2.0 Preparation of 500 mL Complete MesenCult® Proliferation Medium (Mouse):

1. Thaw MesenCult® MSC Proliferation Supplements (Mouse; Catalog #05502) overnight at 2 - 8°C or for several hours at room temperature (15 - 25°C) prior to usage. The supplement can be aliquoted into smaller volumes and stored at -20°C.
2. Add the entire 100 mL MesenCult® MSC Proliferation Supplements (Mouse) to 400 mL MesenCult® MSC Basal Medium (Mouse; Catalog #05501) and mix thoroughly. This is now referred to as Complete MesenCult® Proliferation Medium (Mouse). If less than 500 mL will be required in a 1 month period, smaller volumes can be prepared.

   e.g. Prepare Complete MesenCult® Proliferation Medium (Mouse) by diluting MesenCult® MSC Proliferation Supplements (Mouse) 1/5 with MesenCult® MSC Basal Medium (Mouse). For example, prepare 100 mL Complete MesenCult® Proliferation Medium (Mouse) by adding 20 mL MesenCult® MSC Proliferation Supplements (Mouse) to 80 mL MesenCult® MSC Basal Medium (Mouse).

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3.0 Culture Set-up:

Adipogenic differentiation can be performed on MSCs at different passages, however, cells at earlier passages (e.g. P1 - P4) are recommended.

1. It is recommended to plate mouse mesenchymal stem cells as follows in 2 mL Complete MesenCult® Proliferation Medium (Mouse) per well of a 6-well plate.

	Mouse bone marrow	Mouse compact bone
Fresh unprocessed bone marrow or compact bone cells	7.5-10 x 10^6 cells/well	2-5 x 10^5 cells/well
Passaged bone marrow or compact bone cells	1-3 x 10^5 cells/well	1-1.5 x 10^5 cells/well

2. Culture cells at 37°C at 5% O_2/10% CO_2 for 2 - 5 days until an adherent layer that is 80-90% confluent has formed. Change the medium from Complete MesenCult® Proliferation Medium (Mouse) to Complete MesenCult® Adipogenic Medium (Mouse).

   Note: Ensure that the majority of cells present in the cultures are MSCs and not contaminating macrophages. When using mouse bone marrow-derived MSCs, a high number of macrophages will persist in the culture up until passage 4 when the cells are cultured at 20%O_2/5%CO_2. Refer to the technical manual (Manual Catalog #28374) for images of mouse MSC cultures with macrophage contamination.

3. Culture cells for 2 - 3 weeks at 5% O_2/10% CO_2. If the medium becomes acidic (yellow in color), perform a half-medium change by removing half of the medium and replacing with fresh Complete MesenCult® Adipogenic Medium (Mouse). Adipogenic development will be visible as fat globules in cells in specific areas throughout the culture.

4. Oil Red O (e.g. Millipore Catalog #ECM950) staining may be used to confirm the development of fat globules in adipocytes.

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