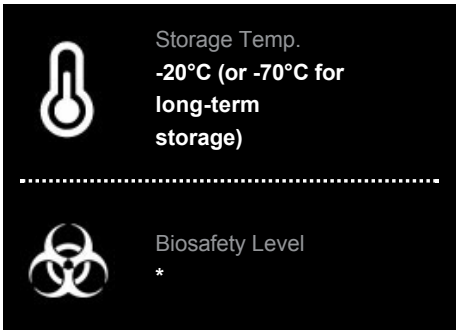
ATCC

Product Sheet

Adipocyte Differentiation Toolkit for Bone Marrow and Umbilical-Derived MSCs (ATCC® PCS-500-053™)

Please read this FIRST



Description

Product Description: The Adipocyte Differentiation Toolkit for Bone Marrow and Umbilical-Derived MSCs (ATCC PCS-500-053) contains medium and reagents designed both to induce adipogenesis in actively proliferating Bone-marrow (ATCC PCS-500-012) and Umbilical-derived (ATCC PCS-500-010) Mesenchymal Stem Cells with high efficiency and to support maturation of derived adipocytes during lipid accumulation.
Volume: 1 kit

Directions for Use

Unpacking and Storage Instructions

1. Check all containers for leakage or breakage.
2. Store the differentiation toolkit at -20°C in a freezer that is not self-defrosting. Do not refreeze the supplements once thawed.
3. If thawed upon arrival, the supplements can be stored at 2°C to 8°C as long as they are added to the Adipocyte Basal Medium within 72 hours.
4. Once prepared, both supplemented media are stable for up to three weeks when stored in the dark at 2°C to 8°C.

Note: Please see the "Adipocyte Differentiation Media Preparation" section before proceeding. Antimicrobials and phenol red are not required but may be added to the 100 mL bottle of Adipocyte Basal Medium if desired prior to supplementation. The recommended volume of each optional component to be added to the Adipocyte Basal Medium is summarized in the table below.

Component	Volume	Final Concentration
Gentamicin-Amphotericin B Solution	0.1 mL	Gentamicin: 10 µg/mL Amphotericin B: 0.25 µg/mL
Penicillin-Streptomycin-Amphotericin B Solution	0.1 mL	Penicillin: 10 Units/mL Streptomycin: 10 µg/mL Amphotericin B: 25 ng/mL
Phenol Red	0.1 mL	33 µM

Preparing Cells for Adipocyte Differentiation

1. Follow the instructions for the growth of Bone Marrow-derived Mesenchymal Stem Cells (ATCC PCS-500-012) or Umbilical-derived MSCs (ATCC PCS-500-010). It is recommended that the cells not be passaged more than four (4) times before initiating adipocyte differentiation.
2. When cells are 70-80% confluent, passage them into a tissue culture plate at a density of 20,000 cells/cm^2 for Bone Marrow-derived MSCs or at 40,000 cells/cm^2 for Umbilical-derived MSCs. Adjust the number of cells and volume of media according to the tissue culture plate used.
3. Example: For a 6 well tissue culture plate with a surface area of 9.5 cm^2/well, add a total of 190,000 cells/cm2 for Bone Marrow-derived MSCs or 380,000 cells/cm^2 for Umbilical-derived MSCs to each well containing 2 mL of Mesenchymal Stem Cell Basal Medium (ATCC PCS-500-030) supplemented with the respective Mesenchymal Stem Cell Growth Kit (ATCC PCS-500-040 or ATCC PCS-500-041) components.
4. Gently rock the plate back and forth and side to side to evenly distribute cells before incubation. Do not swirl.
5. Incubate the cells at 37°C with 5% CO_2 for 48 hours before initiating adipocyte differentiation.

Adipocyte Differentiation Media Preparation

1. Thaw the ADM supplement of the differentiation kit and warm to 37°C in a water bath. Note: It may be necessary to shake the ADM Supplement upon warming to help re-dissolve any components that may have precipitated out of solution upon freezing.
2. Decontaminate the external surfaces of all kit components by spraying them with 70% ethanol.
3. Using aseptic technique and working in a laminar flow hood or biosafety cabinet, add 10 mL of ADM Supplement to 100 mL of Adipocyte Basal Medium.
4. Tightly cap the container of media and swirl the contents gently to assure a homogeneous solution. Do not shake forcefully to avoid foaming. Label and date the bottle.
5. Each container of differentiation medium should be stored in the dark at 2°C to 8°C (do not freeze). When stored under these conditions, the differentiation media is stable for up to six weeks.

Adipocyte Differentiation Procedure

1. After 48 hours gently aspirate the expansion medium from each well and replace with PBS.

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

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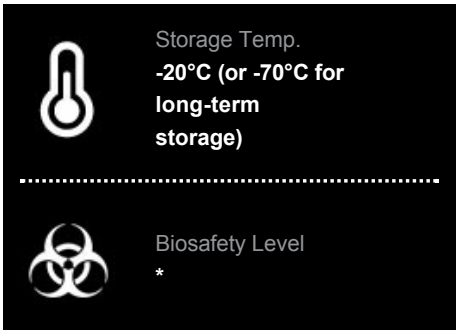

2. Replace PBS with Adipocyte Differentiation Medium.
3. Incubate at 37°C, 5% CO_2
4. Every 3-4 days, gently replace the medium in the wells with fresh Adipocyte Differentiation Medium.
5. Incubate at 37°C, 5% CO_2
6. Between Day 21-35, differentiation should be complete
7. Cells can be used at any phase of adipocyte differentiation as predicated upon experimental design. To confirm lipid accumulation, cells can be fixed and stained with Oil Red O.

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The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

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Additional information on this culture is available on the ATCC web site at www.atcc.org.

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