ATCC

Product Sheet

Adipocyte Differentiation Toolkit for Adipose-Derived MSCs and Preadipocytes (ATCC® PCS-500-050™)

Please read this FIRST

Storage Temp.
-20°C (or -70°C for long-term storage)

Biosafety Level
1

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Description

Product Description: The Adipocyte Differentiation Toolkit (ATCC® PCS-500-050) contains medium and reagents designed both to induce adipogenesis in actively proliferating Adipose-Derived Mesenchymal Stem Cells (ATCC® PCS-500-011) with high efficiency and to support maturation of derived adipocytes during lipid accumulation.

Adipocyte Differentiation Toolkit Components*

1. One vial containing 1 mL of AD Supplement for use in initiation of adipocyte differentiation.
2. One bottle containing 5 mL of ADM Supplement for use in maintaining adipocyte differentiation.
3. One bottle containing 100 mL of Adipocyte Basal Medium for use in both initiation and maintenance of adipocyte differentiation.

**The Adipocyte Differentiation Toolkit provides enough medium and reagents for differentiation of ~6.8 x 10^5 cells when plated at a recommended density of 18,000 viable cells/cm^2 in 4 wells of a 6-well tissue culture format.*

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 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 to 8°C.

Note: Instructions for preparing (1) the Initiation Medium with AD Supplement, and (2) the Maintenance Medium with the ADM Supplement are provided below. 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 Table 1.

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 Adipose-Derived Mesenchymal Stem Cells (ATCC PCS-500-011). 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 18,000 cells/cm^2. 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 171,000 viable cells to each well containing 2 mL of Mesenchymal Stem Cell Basal Medium (ATCC PCS-500-030) supplemented with Mesenchymal Stem Cell Growth Kit–Low Serum (ATCC PCS-500-040) 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

The adipocyte differentiation process requires **two** separate media preparations: one for initiation and one for maintenance. Stock solutions of these media can be prepared in tandem in advance as follows:

1. Thaw all three components of the differentiation kit and warm to 37°C in a water bath. **Note**: It may be

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necessary to shake the AD Supplement and 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 three kit components by spraying them with 70% ethanol.
3. Using aseptic technique and working in a laminar flow hood or biosafety cabinet:
   a. Transfer 15 mL of Adipocyte Basal Medium and 1 mL of AD Supplement to a sterile 50 mL conical tube, using a separate sterile pipette for each transfer. This is your working stock of Adipocyte Differentiation Initiation Medium used during the first 96 hours of differentiation.
   b. Add 5 mL of ADM Supplement to the remaining 85 mL of Adipocyte Basal Medium. This is your working stock of Adipocyte Differentiation Maintenance Medium.
4. Tightly cap the each 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 three weeks.

Adipocyte Differentiation Procedure

Initiation Phase

1. After incubating the prepared Adipose-Derived Mesenchymal Stem Cells for (as described above), carefully aspirate the media from the wells.
2. Immediately rinse the cells once by adding 2 mL of room-temperature D-PBS (ATCC® 30-2200) to each well, then carefully aspirate the PBS from the wells.
3. Add 2 mL of pre-warmed (37°C) **Adipocyte Differentiation Initiation Medium** to each well to begin the adipocyte differentiation process. **Note**: It is recommended that you transfer the required volume of media to a sterile tube for pre-warming prior to each feeding rather than repeatedly re-warming the entire working stock.
4. Incubate the cells at 37°C with 5% CO_2 for 48 hours.
5. Feed the cells by carefully removing half the volume of media (1 mL) from each well and adding another 2 mL of pre-warmed (37°C) **Adipocyte Differentiation Initiation Medium** to each well. **Important**: DO NOT TILT plate during aspiration. It is important that the cell monolayer is not exposed to air during this and subsequent steps to ensure that developing lipid vesicles do not burst.

Maintenance Phase

6. Incubate the cells at 37°C with 5% CO_2 for 48 hours.
7. Carefully remove 2 mL of media from each well (leaving 1 mL) and replace with 2 mL of pre-warmed (37°C) **Adipocyte Differentiation Maintenance Medium** in each well. **Important**: DO NOT TILT plate during aspiration. It is important that the cell monolayer is not exposed to air during this and subsequent steps to ensure that developing lipid vesicles do not burst.
8. Repeat Steps 6 and 7 every 3-4 days for another 11 days until adipocytes reach full maturity. (Full maturity will be reached 15 days after the beginning of initiation phase, or 17 days from initial plating of cells.)
9. 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.

ATCC Warranty

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.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

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

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