Adipocyte Differentiation Toolkit for Adipose-Derived MSCs and Preadipocytes

PCS-500-050™

Description

The Adipocyte Differentiation Toolkit for Adipose-derived MSCs and Preadipocytes contains medium and reagents designed to induce adipogenesis in actively proliferating Adipose-Derived Mesenchymal Stem Cells and Preadipocytes with high efficiency, and to support the maturation of derived adipocytes during lipid accumulation.

Shipping information: 1 kit

Storage Conditions

Product format: Frozen

Storage conditions: -20°C or colder, -70°C for long-term storage

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories*

(BMBL), U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Handling Procedures

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

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		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 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.

Quality Control Specifications

Mycoplasma contamination: Not detected

Functional tests: Differentiation of cells into adipocytes as demonstrated by Oil Red O staining.

A Certificate of Analysis (COA) is available upon request for each lot

Material Citation

If use of this material results in a scientific publication, please cite the material in the

following manner: Adipocyte Differentiation Toolkit for Adipose-Derived MSCs and Preadipocytes (ATCC PCS-500-050)

References

References and other information relating to this material are available at www.atcc.org.

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