Osteocyte Differentiation Tool

PCS-500-052™

Description

The Osteocyte Differentiation Tool is a complete differentiation medium designed to induce osteogenesis in actively proliferating Adipose-derived Mesenchymal Stem Cells (ATCC PCS-500-011) with high efficiency. This product may also be used with Bone Marrow-derived Mesenchymal Stem Cells (ATCC PCS-500-012). The Osteocyte Differentiation Tool provides enough medium for differentiation of ~ 1 million cells when plated at a recommended density of 18,000 viable cells/cm2 in a 6-well tissue culture format.

Volume: 100 mL

Storage Conditions

Product format: Frozen
Storage conditions: -20°C or colder

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

Antimicrobials and phenol red are not required but may be added to the Osteocyte Differentiation Tool if desired prior to use. The recommended volume of each ***optional*** component to be added to Osteocyte Differentiation Tool is summarized in Table 1.

Table 1. Optional Addition of Antimicrobials/Antimycotics and Phenol Red per 100 ml of medium

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

****Please note that the use of phenol red may enhance and accelerate the rate of calcium deposition during osteocyte differentiation.*

Preparing Cells for Osteocyte Differentiation

1. Follow the instructions for the growth of Adipose-Derived Mesenchymal Stem Cells (ATCC® PCS-500-011). Do not passage the cells more than four (4) times before initiating osteocyte differentiation.
2. When cells are 70%-80% confluent, passage them into a tissue culture plate at a density of 18,000 viable cells/cm2. 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 osteocyte differentiation.

Osteocyte Differentiation Procedure

1. After incubating the prepared Adipose-Derived Mesenchymal Stem Cells for 48 hours (as described above), pre-warm the Osteocyte Differentiation Tool to 37°C in a water bath.
2. Bring a bottle of D-PBS (ATCC® 30-2200) to room temperature.
3. Remove the prepared Adipose-Derived Mesenchymal Stem Cells from the incubator and carefully aspirate the culture medium from each well.
4. Rinse the cells by gently adding 2 mL of room-temperature D-PBS (ATCC® 30-2200) to each well, then aspirating the PBS rinse from the wells while being

careful not to disturb the cells.

5. Add 2 mL of the pre-warmed Osteocyte Differentiation Tool to each well. (Store the remaining Osteocyte Differentiation Tool in the dark at 2°C-8°C for later use).
6. Incubate the cells at 37°C with 5% CO2 for 3-4 days before renewing the medium.
7. When ready to renew the medium, retrieve the Osteocyte Differentiation Tool from storage and transfer the required volume to a sterile tube. (For a complete 6-well plate, this volume would be 12 mL).
8. Warm the transferred aliquot of Osteocyte Differentiation Tool to 37°C in a water bath.
9. Remove all but 1 mL of the old medium from each well containing cells.

Important: DO NOT TILT the plate during aspiration or otherwise expose the monolayer to air during this or any subsequent steps.

1. Add 2 mL of fresh, pre-warmed Osteocyte Differentiation Tool to each well by pipetting the medium gently down the side of the well to keep from disturbing the monolayer or accumulated calcium crystals. (This now brings the final volume in each well to 3 mL).

Note: The monolayer of differentiating cells is under tension and extremely fragile. The cells can easily detach from the plate and must be handled with care.

1. Repeat steps 6 through 10 every 3-4 days until the cells have been exposed to the Osteocyte Differentiation Tool for a total of 19 days.
2. Cells can be used at any phase of osteocyte differentiation as predicated upon experimental design. To confirm calcium accumulation, cells can be fixed and stained with Alizarin Red (not provided).

Note: If curling of the edges of the monolayer is observed, the cells will detach from the tissue culture plate within 24-48 hours and should be used immediately.

Quality Control Specifications

Bacterial and fungal testing: Not detected
Mycoplasma contamination: Not detected
Functional tests: Differentiation of cells into osteocytes as demonstrated by Alizarin Red staining.

Osteocyte Differentiation Tool

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A Certificate of Analysis (COA) is available upon request for each lot of the Osteocyte Differentiation Tool.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: Osteocyte Differentiation Tool (ATCC PCS-500-052)

References

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

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