



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.

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:

Osteocyte Differentiation Tool

PCS-500-052

		25 ng/mL
Phenol Red***	0.1 mL	33 μ M

***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 cm²/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% CO₂ 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.

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.

Warranty

The product is provided 'AS IS' and the viability of ATCC[®] products is warranted for 30 days from the date of shipment, provided that the customer has stored and handled the product according to the information included on the product information sheet, website, and Certificate of Analysis. For living cultures, ATCC lists the media formulation and reagents that have been found to be effective for the product. While other unspecified media and reagents may also produce satisfactory results, a change in the ATCC and/or depositor-recommended protocols may affect the recovery, growth, and/or function of the product. If an alternative medium formulation or reagent is used, the ATCC warranty for viability is no longer valid. Except as expressly set forth herein, no other warranties of any kind are provided, express or implied, including, but not limited to, any implied warranties of merchantability, fitness for a particular purpose, manufacture according to cGMP standards, typicality, safety, accuracy, and/or noninfringement.

Disclaimers

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. Any proposed commercial use is prohibited without a license from ATCC.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate or complete and the customer bears the sole responsibility of confirming the accuracy and completeness of any such information.

This product is sent on the condition that the customer is responsible for and assumes all risk and responsibility in connection with the receipt, handling, storage, disposal, and use of the ATCC product including without limitation taking all appropriate safety and handling precautions to minimize health or environmental risk. As a condition of receiving the material, the customer agrees that any activity undertaken with the ATCC product and any progeny or modifications will be conducted in compliance with all applicable laws, regulations, and guidelines. This product is provided 'AS IS' with no representations or warranties whatsoever except as expressly set forth herein and in no event shall ATCC, its parents, subsidiaries, directors, officers, agents, employees, assigns, successors, and affiliates be liable for indirect, special, incidental, or consequential damages of any kind in connection with or arising out of the customer's use of the product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of such materials.

Please see the material transfer agreement (MTA) for further details regarding the use of this product. The MTA is available at www.atcc.org.

Copyright and Trademark Information

© ATCC 2023. All rights reserved.

ATCC is a registered trademark of the American Type Culture Collection.

Revision

Osteocyte Differentiation Tool

PCS-500-052

Product Sheet

This information on this document was last updated on 2023-08-19

Contact Information

ATCC

10801 University Boulevard

Manassas, VA 20110-2209

USA

US telephone: 800-638-6597

Worldwide telephone: +1-703-365-2700

Email: tech@atcc.org or contact your local distributor
