ATCC

Product Sheet

Osteocyte Differentiation Tool (ATCC® PCS-500-052™)

Please read this FIRST

Storage Temp.
-20°C or colder until ready for use. When stored as directed, the product is stable until the expiration date on the label. Once thawed for use, the product is stable for 3 weeks when stored in the dark at 2°C-8°C.

Biosafety Level
1

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

Or contact your local distributor

Description

Product Description: The Osteocyte Differentiation Tool (ATCC® PCS-500-052) is a complete differentiation medium designed to induce osteogenesis in actively proliferating Adipose-Derived Mesenchymal Stem Cells (ATCC® PCS-500-011) with high efficiency.
Formulation: Proprietary

Volume: 100 ml

Directions for Use

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.

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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.

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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