Chondrocyte Differentiation Tool

PCS-500-051™

Description

The Chondrocyte Differentiation Tool is a complete differentiation medium designed to induce chondrogenesis in actively proliferating Adipose-derived Mesenchymal Stem Cells with high efficiency. The Chondrocyte Differentiation Tool provides enough medium to differentiate up to ~ 13.5 x 10^7 cells, sufficient to seed 20 wells of a 48-well tissue culture plate when using alginate encapsulation.

Components: *The Chondrocyte Differentiation Tool provides enough medium for differentiation of ~ 1 million cells when plated at a recommended density of 18,000 viable cells/cm^2 in a 6-well tissue culture format.

Volume: 100 mL

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

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

Table 1. OptionalAddition 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-	0.1 mL	Penicillin: 10

Streptomycin-Amphotericin B Solution		Units/mL Streptomycin: 10 µg/mL Amphotericin B: 25 ng/mL
Phenol Red	0.1 mL	33 µM

Additional Materials Needed for Alginate Encapsulation (not provided):

1. Sodium alginate (e.g., Sigma-Aldrich #71238)
2. 3 mL syringe
3. 27-gauge needle
4. Steriflip® Filter Unit, Millipore Corporation (Vacuum-driven 50 mL filtration system)
5. Wide bore pipette tip
6. 150 mM NaCl solution
7. 100 mM CaCl2 solution (sterile)
8. Small magnetic stir bar (sterile)
9. 250 mL Beaker (sterile)
10. Forceps or stir bar extractor (sterile)
11. Magnetic stir plate

Chondrocyte Differentiation

Chondrocyte differentiation requires that the cells be grown in a three-dimensional aggregate cell culture. Micromass culture can be used; but, for the best results, ATCC recommends the use of a matrix, such as alginate, to provide a scaffold for the deposition of proteoglycans. The following procedure demonstrates differentiation of ~2.7 x 10^7 cells seeded to four wells of a 48-well tissue culture plate, using 25 mL of the Chondrocyte Differentiation Tool and alginate encapsulation.

Preparation of a 1.5% (w/v) Alginate Solution

1. Add 0.15 g of alginate to 10 mL of 150 mM NaCl while stirring rapidly or vortexing to minimize clumping.
2. Agitate the solution on a rocker for at least 2 hr and up to 16 hr at room temperature to completely solubilize the alginate.
3. Sterilize the solution using a 0.22 mm filter. Store at 4°C to 8°C for up to week.

Preparation of Differentiation Medium

1. Pre-warm the Chondrocyte Differentiation Tool to 37°C in a water bath.
2. Once thawed, store the remaining Chondrocyte Differentiation Tool in the dark at 2°C-8°C for later use. When stored under these conditions, the differentiation media is stable for up to three weeks.

Note: For procedures using less than the full 100 mL volume of the Chondrocyte Differentiation Tool, the medium can be dispensed into appropriate aliquots (e.g., for use with this protocol, we recommend dispensing into 25 mL aliquots). Unused portions can be refrozen once without loss of efficiency; however, multiple freeze/thaw cycles are NOT recommended.

Preparing Cells for Chondrocyte Differentiation

1. Follow the instructions for the growth of Adipose-Derived Mesenchymal Stem Cells (ATCC® PCS-500-011) using Mesenchymal Stem Cell Basal Medium (ATCC® PCS-500-030) supplemented with Mesenchymal Stem Cell Growth Kit – Low Serum (ATCC® PCS-500-040) components.
2. Expand the cells as needed for experimental design, but do not passage more than four (4) times prior to initiating chondrocyte differentiation.

Note: It will take approximately 10 to 15 T-75 flasks at 70%-80% confluence to obtain 2.7 x 10^7 cells, which is enough to seed four wells of a 48-well tissue culture plate using the alginate encapsulation method described below.

1. Cells should be collected and counted when the culture is 70%-80% confluent and actively proliferating.
2. After centrifugation at 150 x g for 3-5 minutes, discard the supernatant and resuspend the cell pellet (2.5 x 107 cells) in 800 µL of the 1.5% (w/v) Alginate Solution. The final volume should be ~1.0 mL.

Note: The Alginate Solution must not be diluted lower than 1.2% (w/v) by the addition of the cells. If you have fewer cells, adjust the volume of the prepared 1.5% Alginate Solution used in order to maintain this ratio. Likewise, if you have more cells, scale proportionally.

1. Gently mix the cell-alginate suspension by pipetting up and down; taking care not to introduce bubbles into the solution.
2. Proceed to Step 1 (below) for alginate encapsulation of cells.

Alginate Encapsulation of Cells: Formation of Chondrogenic Microbeads

1. Transfer 75 mL of a 100 mM sterile CaCl2 solution to a sterile 250 mL beaker containing a sterile stir bar.
2. Create a gentle funnel in the CaCl2 solution at room temperature on a stir plate.
3. Transfer the alginate-cell suspension with a 3 mL syringe fitted with a 27 gauge needle.
4. Rapidly dispense the alginate-cell suspension into the CaCl2 solution to form chondrogenic microbeads.
5. Allow the chondrogenic microbeads to stir for 10 minutes to solidify (cure) the alginate.
6. Remove the beaker from the stir plate and allow the chondrogenic microbeads to settle on the bottom.
7. Transfer the chondrogenic microbead solution into a 50 mL conical tube and attach to the vacuum-driven Steriflip® Filter Unit. Immediately break the vacuum as soon as the liquid is removed to prevent damage to the beads.
8. Resuspend the chondrogenic microbeads in 2 mL of pre-warmed Chondrocyte Differentiation Tool.
9. Aseptically transfer enough chondrogenic microbeads to cover the bottom surfaces of four wells of a 48-well plate (about 0.5 mL per well). This set up will yield ~6.75 x 10^6 encapsulated cells/well.
10. Allow the chondrogenic microbeads to settle to the bottom of the wells. To remove residual CaCl2, wash the chondrogenic microbeads by replacing the medium in each well twice with 0.5 mL Chondrocyte Differentiation Tool. Add

0.5 mL Chondrocyte Differentiation Tool to each well after the last wash.

11. Incubate the cells at 37°C with 5% CO2 for 2-3 days before renewing the medium.
12. When ready to renew the medium, retrieve the Chondrocyte Differentiation Tool from storage and transfer the required volume to a sterile tube. (For 4 wells in a 48-well plate, this volume would be 2 mL).
13. Warm the aliquot of Chondrocyte Differentiation Tool to 37°C in a water bath.
14. Carefully remove the spent medium, taking great care not to disturb or aspirate the chondrogenic microbeads.
15. Add 0.5 mL of fresh, pre-warmed Chondrocyte Differentiation Tool to each well.
16. Incubate the cells at 37°C with 5% CO_2 for 2-3 days before renewing the medium.
17. Repeat steps 12 through 16 every 2-3 days until the cells have been exposed to the Chondrocyte Differentiation Tool for a total of 21 days.
18. Cells can be used at any phase of chondrocyte differentiation as predicated upon experimental design. To confirm calcium accumulation, cells can be fixed and stained with Alcian Blue (not provided).

Quality Control Specifications

Bacterial and fungal testing: Not detected
Mycoplasma contamination: Not detected
Functional tests: Differentiation of cells into chondrocytes as demonstrated by Alcian Blue staining.
A Certificate of Analysis (COA) is available upon request for each lot of the Chondrocyte Differentiation Tool.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: Chondrocyte Differentiation Tool (ATCC PCS-500-051)

References

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

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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