C20A4 Human Chondrocyte Cell Line

Immortalized Cell Line

Cat. # SCC041

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
NOT FOR HUMAN OR ANIMAL CONSUMPTION
THIS PRODUCT CONTAINS GENETICALLY MODIFIED ORGANISMS

Pack size: ≥1X10^6 viable cells/vial

Store in liquid nitrogen

Certificate of Analysis

Background

The chondrocyte is the only specialized cell type present in articular cartilage. They produce secretions to support and repair the cartilage matrix. Human chondrocytes in mature articular cartilage are post-mitotic and terminally differentiated cells. Studies on human chondrocytes have been hampered by the difficulty in obtaining sufficient numbers of primary human chondrocytes from a single joint and by variabilities among donors due to factors such as age and medical conditions.

C20A4 (alternatively known as C-20/A4) is an immortalized human chondrocyte line that is widely used as a model cell line for studying normal and pathological cartilage repair mechanisms related to chondrocyte biology and physiology.

Short Tandem Repeat (STR) Profile

D3S1358: 17, 18	D16S539: 12, 13
TH01: 6, 9.3	CSF1PO: 10, 12
D21S11: 29, 30	Penta D: 11, 12
D18S51: 12, 21	vWA: 16, 17
Penta E: 7, 16	D8S1179: 13
D5S818: 11, 13	TPOX: 10, 12
D13S317: 8, 12	FGA: 21
D7S820: 9	Amelogenin: X, Y

Immortalized cell lines are inherently genetically unstable. Genetic instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passages.

Source

C-20/A4 cell line was established by transfecting primary cultures of rib chondrocytes from a 5-year-old male with vectors expressing origin-defective simian virus SV40 large T antigen.

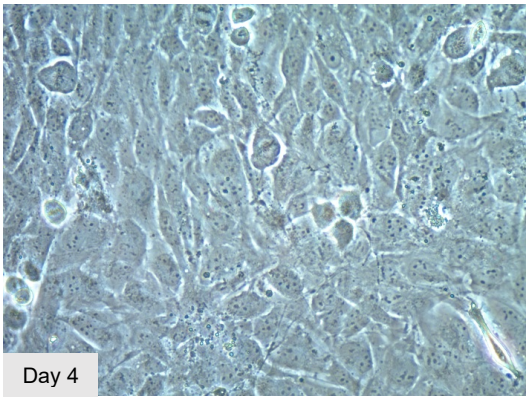
Quality Control Testing

- Each vial contains ≥1X10^6 viable cells.
- Cells are tested negative for HPV-16, HPV-18, Hepatitis A, B, C, and HIV-1 & 2 viruses by PCR.
- Cells are negative for mycoplasma contamination.
- Each lot of cells is genotyped by STR analysis to verify the unique identity of the cell line.

Storage and Handling

C-20/A4 human chondrocytes should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Data



References

1. Goldring, M.B, Birkhead, J.R., Suen, L.F., Yamin, R., Mizuno, S., Glowacki, J., Arbiser, J.L, Apperley, J.F. (1994) Interleukin-1 beta-modulated gene expression in immortalized human chondrocytes. *J. Clin. Invest* 94(6): 2307-2316.

2. Finger, F., Schorle, C., Soder, S., Zien, A., Goldring, M.B., Aigner, T. (2004) Phenotypic characterization of human chondrocyte cell line C-20/A4: a comparison between monolayer and alginate suspension culture. *Cells Tissues Organs*. 178(2): 65-77.

3. Goldring, M.B. (2004) Culture of immortalized chondrocytes and their use as models of chondrocyte function. *Methods Mol. Med*. 100: 37-52.

SPECIES LEGEND: **H** Human **Ca** Canine **M** Mouse **R** Rat **Rb** Rabbit **B** Bovine **P** Porcine **WR** Most Common Vertebrates

Please visit www.millipore.com for additional product information and references.
Submit your published journal article, and earn credit toward future purchases. Visit www.millipore.com/publicationrewards to learn more!

Ver.2.0/2017-09-07/SCC041/AN

Protocols

Thawing Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on normal tissue culture ware surfaces without any additional coating.

   Cells are thawed and expanded in DMEM High Glucose (EMD Millipore Cat. No. SLM-120-B) containing 10% FBS (EMD Millipore Cat. No. ES-009-B),
2. Remove the vial of frozen C-20/A4 cells from liquid nitrogen and incubate in a 37°C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

   IMPORTANT: Do not vortex the cells.
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of 10% FBS Media (Step 1 above; pre-warmed to 37°C) to the 15 mL conical tube.

   IMPORTANT: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   IMPORTANT: Do not vortex the cells.
7. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 10-15 mL of 10% FBS Medium (pre-warmed to 37°C).
10. Transfer the cell mixture to a T75 tissue culture flask.
11. Incubate the cells at 37°C in a humidified incubator with 5% CO2.
12. The next day, exchange the medium with 10-15 mL of fresh 10% FBS Medium pre-warmed to 37°C. Exchange with fresh medium every two to three days thereafter.
13. When the cells are approximately 90% confluent, they can be dissociated with Accutase (EMD Millipore Cat. No. SCR005) or trypsin-EDTA (EMD Millipore Cat. No. SM-2003-C) and further passaged or, alternatively, frozen for later use.

Subculturing Cells

1. Carefully remove the medium from the T75 tissue culture flask containing the confluent layer of C-20/A4 cells.
2. Apply 3-5 mL of Accutase or trypsin-EDTA solution and incubate in a 37°C incubator for 3-5 minutes.
3. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
4. Add 8 mL of 10% FBS Medium (pre-warmed to 37°C) to the plate.
5. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
6. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.
7. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
8. Apply 2 mL of 10% FBS Medium (pre-warmed to 37°C) to the conical tube and resuspend the cells thoroughly.

   IMPORTANT: Do not vortex the cells.
9. Count the number of cells using a hemocytometer.
10. Plate the cells to the desired density (typical split ratio is 1:8 to 1:10).

Cryopreserving Cells

C-20/A4 cells can be frozen in the expansion media plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

ACADEMIC USE AGREEMENT
(subject to local law)

THIS PRODUCT MAY ONLY BE USED BY INDIVIDUALS EMPLOYED BY AN ACADEMIC INSTITUTION AND IS INTENDED SOLELY TO BE USED FOR ACADEMIC RESEARCH, WHICH IS FURTHER DEFINED BELOW. BY OPENING THIS PRODUCT, YOU ("PURCHASER") HEREBY REPRESENT THAT YOU HAVE THE RIGHT AND AUTHORITY TO LEGALLY BIND YOURSELF AND/OR YOUR EMPLOYER INSTITUTION, AS APPLICABLE, AND CONSENT TO BE LEGALLY BOUND BY THE TERMS OF THIS ACADEMIC USE AGREEMENT. IF YOU DO NOT AGREE TO COMPLY WITH THESE TERMS, YOU MAY NOT OPEN OR USE THE PRODUCT AND YOU MUST CALL MILLIPORESIGMA ("SELLER") CUSTOMER SERVICE (1-800-645-5476) TO ARRANGE TO RETURN THE PRODUCT FOR A REFUND.

"Product" means C20A4 Human Chondrocyte Cell Line (SCC041)

"Academic Research" means any internal *in vitro* research use by individuals employed by an academic institution. Academic Research specifically excludes the following uses of whatever kind or nature:

- Re-engineering or copying the Product
- Making derivatives, modifications, or functional equivalents of the Product
- Obtaining patents or other intellectual property rights claiming use of the Product
- Using the Product in the development, testing, or manufacture of a Commercial Product
- Using the Product as a component of a Commercial Product
- Reselling or licensing the Product
- Using the Product in clinical or therapeutic applications including producing materials for clinical trials
- Administering the Product to humans
- Using the Product in collaboration with a commercial or non-academic entity

"Commercial Product" means any product intended for: (i) current or future sale; (ii) use in a fee-for-service; or (iii) any diagnostic, clinical, or therapeutic use.

Access to the Product is limited solely to those officers, employees, and students of PURCHASER's academic institution who need access to the Product to perform Academic Research. PURCHASER shall comply with all applicable laws in its use and handling of the Product and shall keep it under reasonably safe and secure conditions to prevent unauthorized use or access.

These use restrictions will remain in effect for as long as PURCHASER possesses the Product.

COMMERCIAL OR NON-ACADEMIC ENTITIES INTERESTED IN PURCHASING OR USING THE PRODUCT MUST CONTACT licensing@emdmillipore.com AND AGREE TO SEPARATE TERMS OF USE PRIOR TO USE OR PURCHASE.

GMO
This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient des organismes génétiquement modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.