Product sheet

MC3T3-E1 Subclone 14 Cells | 305185

General information

Description	MC3T3-E1 Subclone 14 cells are a valuable resource in biological science, specifically in the study of osteoblasts. Derived from a C57BL/6 mouse calvaria, these cells were carefully selected based on their high alkaline phosphatase (ALP) activity while resting. This unique characteristic makes them an ideal model for investigating osteoblast differentiation and the formation of calcified bone tissue in vitro. As a preosteoblast cell type, MC3T3-E1 Subclone 14 cells exhibit a fibroblast morphology and are primarily associated with bone tissue derived from the calvaria. One of the notable features of MC3T3-E1 Subclone 14 cells is their ability to differentiate into osteoblasts and osteocytes. Through their extensive morphological and functional resemblance to primary calvarial osteoblasts, these cells offer a reliable platform for studying the extracellular matrix (ECM) signalling and behaviour associated with osteoblast differentiation. When cultured with ascorbic acid and inorganic phosphate at optimal concentrations (3 to 4 mM), MC3T3-E1 Subclone 14 cells exhibit remarkable levels of osteoblast differentiation. After just ten days, they form a well-mineralized ECM, providing researchers with a window into the intricate process of bone tissue formation. Moreover, these cells have been found to secrete collagen, an essential component of bone tissue, and express murine leukaemia inhibitory factor (MIF) in RNA. Such characteristics further contribute to their relevance in investigating various biological processes related to bone development and homeostasis. The MC3T3-E1 Subclone 14 cell line has also been employed in cutting-edge research. For instance, it has been utilized to propose an actin filament cytoskeleton analysis framework, offering insights into the complex intracellular architecture of osteoblasts. Additionally, researchers have explored the effects of biodegradable magnesium and magnesium alloys on these cells, studying their interactions with different materials and their impact on selected cellular properties. With their diverse applications, these cells are invaluable in 3D cell culture studies, providing a realistic in vitro model for investigating osteoblast behaviour and differentiation within a three-dimensional environment. Their relevance extends to various research fields, including tissue engineering, bone regeneration, and the development of therapeutic interventions for bone-related disorders.
Organism	Mouse
Tissue	Bone, calvaria
Applications	3D cell culture, Differentiation studies
Synonyms	MC3T3-E1 SUBCLONE 14

Characteristics

Breed/Subspecies	C57BL/6

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Age	Newborn
Gender	Unspecified
Morphology	Fibroblast
Growth properties	Adherent

Regulatory Data

Citation	MC3T3-E1 Subclone 14 (Cytion catalog number 305185)
Biosafety level	1
NCBI_TaxID	10090
CellosaurusAccession	CVCL_5437

Biomolecular Data

Protein expression	Collagen
Tumorigenic	Yes

Handling

Culture Medium	Alpha MEM, w: 2.0 mM stable Glutamine, w: Ribonucleosides, w: Deoxyribonucleosides, w: 1.0 mM Sodium pyruvate, w: 2.2g/L NaHCO3, w/o: Ascorbic acid (GIBCO, Catalog No. A1049001. We do not supply this product; please consider other suppliers. Please let us know if you need further assistance.)
Supplements	Supplement the medium with 10% FBS
Dissociation Reagent	Accutase

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Subculturing	Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium.
Fluid renewal	2 to 3 times per week
Freeze medium	As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

Thawing and Culturing Cells

1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
2. Upon receipt, either store the cryovial immediately at temperatures below -150°C to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
3. For immediate culturing, swiftly thaw the vial by immersing it in a 37°C water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
5. Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
6. Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
7. Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

Incubation Atmosphere	37°C, 5% CO_2, humidified atmosphere.
Flask Coating	None

Shipping Conditions	Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately −78 °C throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.
Storage Conditions	For long-term preservation, place vials in vapor-phase liquid nitrogen at about −150 to −196 °C. Storage at −80 °C is acceptable only as a short interim step before transfer to liquid nitrogen.

Quality Control & Molecular Analysis

Sterility	Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods. To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.