

## **User Manual**

**OriCell™ MC3T3-E1 Subclone 14**

**Mouse Calvarial Preosteoblast**

**Subclone 14 Cell Line with GFP**

**Catalog No. M7-0203**



## Introduction

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The MC3T3-E1 Subclone 14 (mouse calvarial pre-osteoblast) is a clonal cell line derived from the phenotypically heterogeneous parental MC3T3-E1 cell population. This subclone was specifically selected from osteoblasts cultured in the presence of ascorbic acid for its high capacity for osteoblastic differentiation and mineralization.

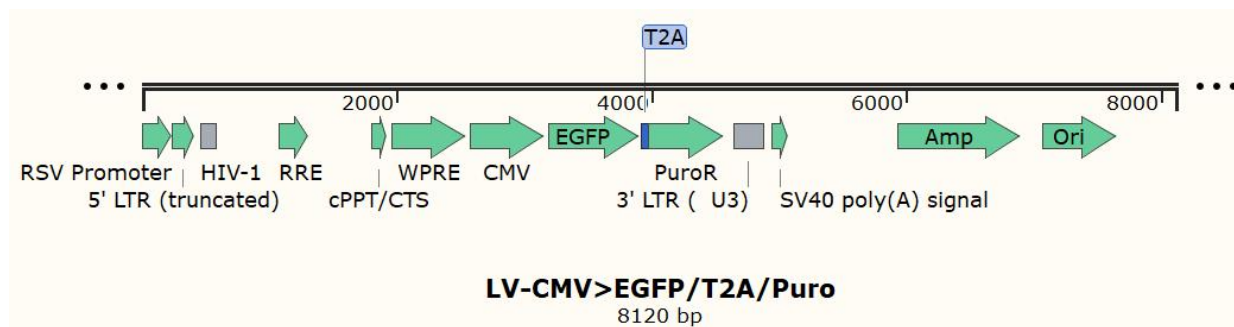
OriCell™ MC3T3-E1 Subclone 14 Mouse Calvarial Preosteoblast Subclone 14 Cell Line with GFP is generated by infecting the parental cells with a recombinant lentiviral vector carrying the GFP gene, resulting in stable expression of green fluorescent protein. This cell line exhibits robust osteoblastic differentiation when cultured with ascorbic acid and 3–4 mM inorganic phosphate, and is capable of forming a well-mineralized extracellular matrix (ECM) after 10 days of culture. It enables convenient cell tracking and visualization both in vitro and in vivo.

In addition, MC3T3-E1 Subclone 14 cells express mRNA for osteoblast-specific markers, including bone sialoprotein (BSP), osteocalcin (OCN), and the parathyroid hormone/parathyroid hormone-related protein receptor (PTH/PTHrP receptor). It has been reported that when implanted into immunodeficient mice, these cells can form bone-like tissue resembling woven bone. This cell line, particularly the ECM it produces, serves as an excellent in vitro model for studying osteoblast differentiation.

**Note:** This product is intended for research use only and is not for diagnostic, therapeutic, clinical, household, or any other applications.

When citing our products in academic publications, please use the following format: “OriCell™ [Product Name] + [Catalog Number], from Cyagen Biosciences.”

## Expression Vector

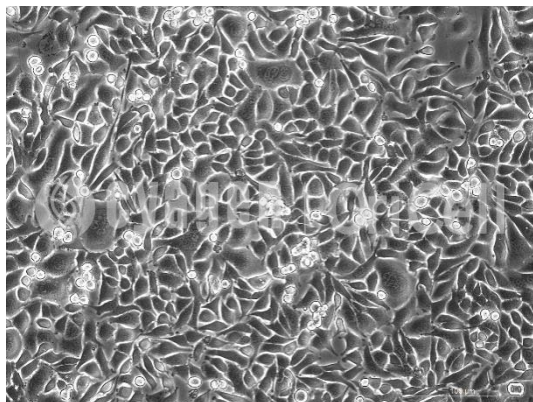


## Product Information

Product Name	OriCell™ MC3T3-E1 Subclone 14 Mouse Calvarial Preosteoblast Subclone 14 Cell Line with GFP
Alternative Name	MC3T3-E1 Subclone 14 with GFP
Catalog Number	M7-0203
Amount of Cells	1×10 <sup>6</sup> cells/vial
Tissue Origin	Mouse Calvaria
Cell Characteristics	Adherent Growth; Fibroblast-like
Culture Conditions	95% air; 5% CO <sub>2</sub> ; 37 °C
Culture Medium	MEM · α + 10% FBS
Doubling Time	12 ~ 24 h
Biosafety Level	1
Storage at	Liquid Nitrogen (-196 °C)
Precautions	Fluorescence Selection: If GFP fluorescence is weak, add puromycin (0.5–2 µg/mL) for 2–4 days every 3–5 passages. Stop selection once fluorescence visibly increases.

**Note:** This product is manufactured under strict aseptic conditions. You may choose to add antibiotics during subsequent culturing based on your specific needs.

## The Morphology of OriCell™ MC3T3-E1 Subclone 14 Cell Line with GFP



### QC

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- Pass the detection of bacteria, fungi, mycoplasma, and endotoxins.
- Verified by cell recovery viability testing.
- Verified by STR analysis.

Please refer to "COA" for details.

### General Handling Principles

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1. Maintain strict aseptic technique. Ensure complete sterility throughout all procedures, particularly within the laminar flow hood and incubator.
2. Follow standardized protocols. Adhere strictly to the product manual. Implement rigorous control over experimental variables and include appropriate parallel controls.
3. Use high-quality consumables and reagents. This product requires culture vessels suitable for adherent growth, and the reuse of these vessels is not recommended. The reagents used must be validated for reliability, cell compatibility, and batch-to-batch consistency.

**Note:** The cryopreservation medium of this product contains DMSO, which may pose potential risks.

Please handle it with care.

Abbreviation	Name	Cat. No.
FBS	Fetal Bovine Serum	See official website
BCS	Bovine Calf Serum	SBCST-01001
Glu	Glutamine	SGLU-10201
SP	Sodium Pyruvate	SCSP-10301
Dex	Dexamethasone	SDEX-10401
NBCS	Newborn Calf Serum	NCSST-01001
HS	Horse Serum	SCHST-01001
NEAA	Non Essential Amino Acid	NEAA-10201
β-mer	β-mercaptoethanol	BMER-10301
P/S	Penicillin- Streptomycin	ATPS-10001
ITS	Insulin / Transferrin / Selenite	ITSS-10201

## Thawing and Culturing of Cells

### Materials Required

- OriCell™ MC3T3-E1 Subclone 14 Mouse Calvarial Preosteoblast Subclone 14 Cell Line with GFP (Cat. No.: M7-0203)
- OriCell™ Complete Medium For MC3T3-E1 Subclone 14 Cell Line (Cat. No.: CMM7-0201)

### Steps

**Note:** If thawing is planned within 24 hours of receipt, store the cells in an ultra-low temperature freezer at -80 °C. For long-term storage (>24 hours), keep them in liquid nitrogen. Before thawing, transfer the cells from liquid nitrogen to -80 °C and hold them there for 10 minutes. This will allow any

residual liquid nitrogen to evaporate and prevent vial explosion.

1. Pre-warm the water bath to 37 °C.
2. Warm the complete medium to 37 °C.
3. Add at least 5 mL of pre-warmed complete medium to a 15 mL centrifuge tube for subsequent use.
4. Remove the cryovial containing cells from the -80 °C freezer, immerse it in the 37 °C water bath, and gently and quickly swirl to thaw the cryopreservation medium.

**Note:**

(1) Gently swirl the cryovial during thawing to ensure rapid and uniform thawing.

(2) Avoid submerging the cap in water to prevent contamination.

(3) Stop thawing in the water bath when only a single ice crystal (approximately 2 mm in diameter) remains, then continue gently swirling the vial until it is completely thawed.

5. Wipe the outer surface of the cryovial with 75% ethanol.
6. In a biosafety cabinet, open the cryovial and transfer the cell suspension to the prepared centrifuge tube using a Pasteur pipette.
7. Rinse the cryovial once with 1 mL of complete medium to collect residual cells and minimize loss.
8. Centrifuge the cell suspension at  $250 \times g$  for 4 minutes.

**Note:** Please calculate the corresponding rotational speed using the formula:  $RCF = 1.118 \times 10^{-5} \times r \times RPM^2$  (where RCF is the relative centrifugal force,  $r$  is the rotor radius in cm, and RPM is the rotational speed).

9. Carefully remove the supernatant after centrifugation. Add 2 mL of complete medium, gently resuspend the cell pellet by pipetting up and down to mix thoroughly.
10. Seed the cells into a T25 flask or culture vessel with an equivalent growth surface area. Add sufficient complete medium so that the total volume in a T25 flask is no less than 5 mL.

11. Gently swirl the flask to evenly distribute the cells, then incubate in a CO<sub>2</sub> incubator at 37 °C with 5% CO<sub>2</sub> and saturated humidity.

**Note:** Do not move or observe the cells within the first 2 hours after seeding, as this may impair cell adhesion, causing poor morphology, clumping, and uneven attachment.

12. On the day after recovery, observe cell status and either replace the medium with fresh complete medium or passage the cells as needed.

**Note:** If an excessive number of floating cells or any abnormal conditions are observed, investigate promptly and contact us for assistance.

13. Replace the complete medium every 3 days until the cells reach approximately 95% confluence, at which point they are ready for passage.

## Passaging of Cells

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### Materials Required

- OriCell™ 0.25% Trypsin-0.04% EDTA Solution (Cat. No.: TEDTA-10001)
- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No.: PBS-10001)
- OriCell™ Complete Medium For MC3T3-E1 Subclone 14 Cell Line (Cat. No.: CMM7-0201)

### Steps

1. Pre-warm the complete medium, PBS and trypsin to 37 °C.
2. Remove the medium in the culture vessel.
3. Gently wash the cells twice with PBS (approximately 3 mL for a T25 flask or 6 mL for a T75 flask). Ensure thorough washing but avoid excessive force. Then remove the PBS.
4. Add trypsin (approximately 1.5 mL for a T25 flask or 3 mL for a T75 flask), quickly spread it to

ensure full coverage of the cell layer.

5. Observe the cells under a microscope. When approximately 70% to 80% of the cells have shrunk and become round, gently tap the outer wall of the culture vessel to detach the cells from the surface.
6. Immediately add complete medium (approximately 3 mL for a T25 flask or 6 mL for a T75 flask), then gently swirl the culture vessel to mix the medium and trypsin, stopping the digestion process.
7. Collect the cell suspension using a pipette, gently pipetting along the bottom of the vessel several times to ensure maximal recovery of the cells.

**Note:** Pipetting should be performed gently to avoid creating excessive bubbles, as this may cause cell damage or loss.

8. Transfer the cell suspension to a centrifuge tube. Rinse the culture vessel once with PBS (approximately 3 mL for a T25 flask and 6 mL for a T75 flask) to collect residual cells and add the wash to the centrifuge tube.
9. Centrifuge all collected cell suspensions at  $250 \times g$  for 4 minutes.
10. Carefully remove the supernatant after centrifugation. Add 2 mL of complete medium and gently resuspend the cell pellet by pipetting up and down to thoroughly mix.
11. Seed the cells into a suitable culture vessel at a density of  $(2-3) \times 10^4$  viable cells/cm<sup>2</sup>, or adjust the seeding density based on the actual growth conditions of the cells.

**Note:** We recommend manual cell counting when conditions permit and counting efficiency is high, in order to obtain an accurate cell concentration to guide seeding. If precise counting is not feasible, subculturing at an appropriate ratio is a better alternative. Typically, MC3T3-E1 Subclone 14 cells with GFP are passaged at a ratio of 1:3 to 1:4, with cells reaching passage confluence within 48 hours. Please adjust the subculture ratio according to the actual condition of the cells.

12. Gently agitate the vessel to ensure uniform cell distribution and place it in an incubator at

37 °C, 5% CO<sub>2</sub>, and saturated humidity.

13. On the day after passaging, observe the cell condition. If a significant number of floating cells are present, replace the culture medium.
14. Replace the culture medium every 3 days. When cells reach 95% confluence, passage or cryopreserve the cells.

## Cryopreservation of Cells

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### Materials Required

- OriCell™ NCR Protein-Free Cryopreservation Medium For General Use (Cat. No.: NCPF-10001)
- OriCell™ NCR Cryopreservation Medium For General Use (Cat. No.: NCRC-10001)

### Steps

1. Cells should be cryopreserved once they reach an appropriate density suitable for passaging.
2. For cell digestion, please refer to passaging steps 1-9 above.
3. Carefully remove the supernatant after centrifugation and gently resuspend the cells in an appropriate volume of cryopreservation medium.
4. Aliquot the cells into cryovials according to the desired cell number or proportion.

**Note:** If accurate cell counting is not feasible, we recommend aliquoting cells proportionally for freezing. Prolonged exposure to non-culture conditions will significantly compromise cell viability. Maintain the cells at 4 °C during counting to minimize metabolic activity and preserve cell integrity.

5. When using any of the recommended NCR cryopreservation media above, cryovials can be directly placed individually into a -80°C freezer.

**Note:** Avoid opening the freezer door during the first 4 hours of freezing, as temperature

fluctuations can adversely affect cell viability.

6. After approximately 8 hours, transfer the cryovials to liquid nitrogen for long-term storage.

**Note:** Do not store the cryovials at -80 °C for more than 48 hours.

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