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IMMORTALIZED CELL LINES

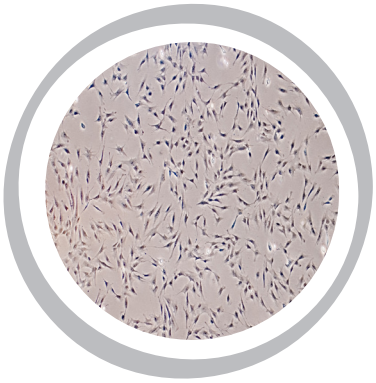
P10979-IM

Bone is a dynamic tissue that undergoes continuous remodeling through the coordinated actions of osteoclasts and the osteoblast lineage. Osteoblasts, the bone-forming cells, originate from pluripotent mesenchymal stem cells. These cells are responsible for synthesizing and secreting the organic extracellular matrix, known as osteoid, which consists primarily of type I collagen. The osteoid is subsequently mineralized by osteoblasts. During this process, osteoblasts become embedded in lacunae within the calcified matrix and differentiate into osteocytes.

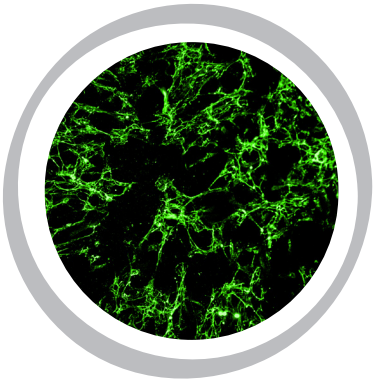
Osteoblasts are known to express protease-activated receptor-1 (PAR-1) and vascular endothelial growth factor (VEGF), both of which play essential roles in bone physiology. Furthermore, research indicates that leukemia inhibitory factor (LIF) can bind to the osteoblast cell surface, stimulating bone formation both in vitro and in vivo. Should you require further elucidation or have specific inquiries, please do not hesitate to express them.

IMMORTALIZED HUMAN FEMORAL OSTEOBLASTS

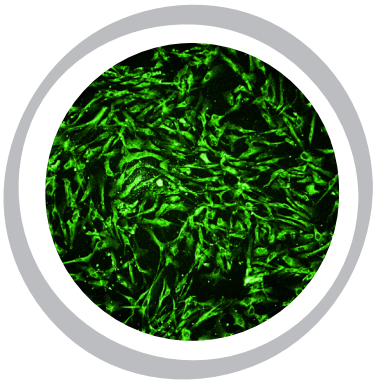
Product Type: Immortalized Human Femoral Osteoblasts
Catalog Number: P10979-IM
Immortalization: SV40 Large T Antigen. G418 resistant.
Number of cells: >1x10^6 cells (cryopreserved vials)
Storage: Liquid Nitrogen
Source: Human healthy femur
Recommended Medium: Osteoblast Medium (Reference: P60119).
Product Characterization: Immunofluorescent method for Collagen, Osteocalcin and Phosphatase A1.



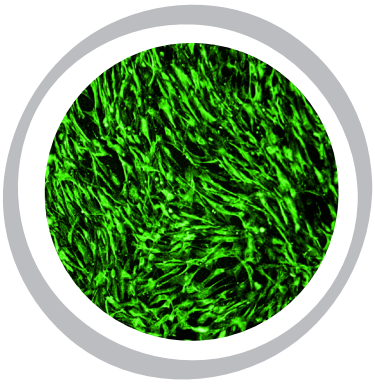
Phase-contrast



Collagen



Phosphatase A1



Osteocalcin

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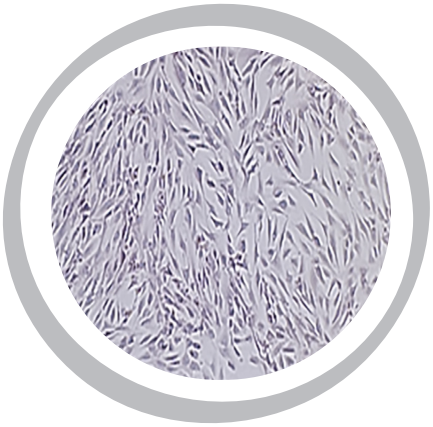
About Immortalized Human Femoral Osteoblasts

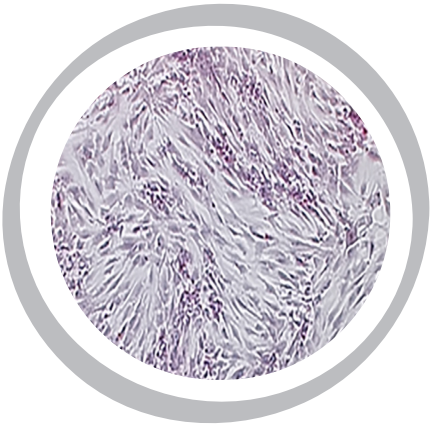
The immortalized human femoral osteoblasts cell line has been developed through genetic modification of primary human femoral osteoblasts, employing the SV40LT protein as the immortalization method. The SV40LT protein, derived from the Simian Virus 40 Large T-antigen, has been introduced to confer immortality to the cells, allowing for an extended lifespan compared to primary cells that typically undergo senescence after a limited number of passages. The use of SV40LT for immortalization is a common technique in cell biology and allows for the establishment of cell lines with a more stable and prolonged growth capacity. This modification enables researchers to conduct long-term experiments and studies that require consistent cellular behavior over an extended period. Primary cells exhibit senescence following the 5th passage, whereas the SV40LT-transduced cells demonstrate a prolonged viability, extending beyond 20 passages.

Functional Analysis: Mineralization Assay

Cells were seeded in 24-well plates at a density of 21000 cells/well in osteoblast medium. On day 2, the medium was replaced with differentiation medium (DMEM low glucose + 10% FBS, 50 µg/ml ascorbic acid, 10 nM beta-glycerophosphate, 10 nM dexamethasone). The medium was changed twice per week for at least 2 weeks. After 2 weeks, the cells were fixed with 4% PFA for 15 minutes and stained with Alizarin Red for 2 hours in an incubator with agitation. Red mineral deposits were observed in cells treated with the differentiation medium.



Control



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Culturing conditions

1 IMMEDIATELY UPON DELIVERY

1.1 Remove the vial from the shipping container to check for freezing.

1.2 Transfer the frozen vial to liquid nitrogen until ready to thaw.

2 THAWING CELLS:

2.1 Prepare "Thawing medium" by combining 500 ml of basal medium, 25 ml of fetal bovine serum, 5 ml of Growth supplement and 5 ml of penicillin/streptomycin solution.

2.2 Thaw cells rapidly in a 37°C water bath; avoid allowing the sample to warm to 37°C. Cryovials should be cool to the touch when removed.

2.3 Remove the vial, wipe it dry, and transfer it to a sterile field.

2.4 Rinse the vial with 70% ethanol, then wipe to remove excess. Open the vial and resuspend its contents using a 1 ml Eppendorf pipette.

2.5 Dispense the contents into a 25 cm^2 culture flask with warm complete media (FBS percentage can be increased up to 10% for better culture establishment).

2.6 Place the flask in the incubator.

2.7 For optimal results, avoid disturbing the culture for 16 hours after initiation. Change the growth medium the next day to remove unattached cells, then every other day thereafter.

3 MAINTENANCE OF THE CULTURE:

3.1 Change medium 48 hours after establishing a subculture.

3.2 Subculture when cells are over 90% confluent.

4 SUBCULTURING:

Remove medium, rinse with 0.05% trypsin-EDTA solution. Add 1 to 2 mL of trypsin-EDTA solution and allow the flask to sit until cells detach. Add fresh culture medium, aspirate, and dispense into new culture flasks. Recommended subcultivation ratio of 1:2 to 1:6. Medium Renewal: 2 to 3 times per week. Reagents for cryopreservation: Cryostor S10.

Quality Control / Biosafety

The cells test negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi.

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