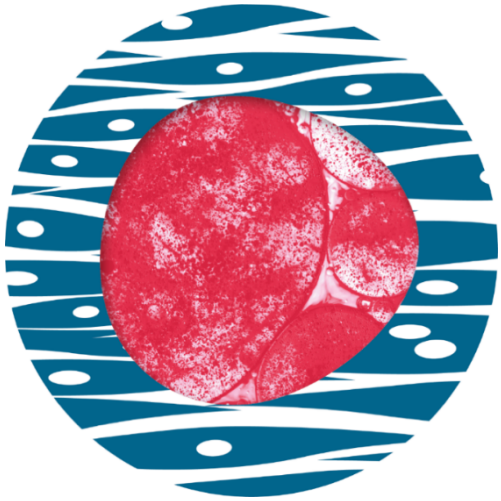
CI-huOB

Immortalized human osteoblast cell line

Product Sheet

→ At a glance		
BSL Level 1	**Coating** Not required	**Growth** Adherent
Storage <2d: -80°C >2d: liquid Nitrogen	**Medium** huOB Maintenance Medium (INS-ME-1006) huOB Differentiation Medium (INS-ME1007)	**Cat.-No.** INS-CI-1005



→ Contents

→ Intended Use and Licensing 2

→ Background Information 3

→ Morphology 3

→ Cell Characterization and Assays 4

  → Marker Expression 4

  → Established Assays 4

→ Quality Control 4

→ Related Products 4

→ Upon Arrival 5

→ Medium Storage and Preparation 5

→ Thaw Cryopreserved Cells 7

→ Freeze Cells for Cryopreservation 8

→ Routine Cell Culture 9

→ References 9

inscreenex **GmbH**
Inhoffenstr. 7
38124 Braunschweig
Germany

→ General Inquiries
M. info@inscreenex.com
T. +49 531 6181 5080

→ Ordering
M. orders@inscreenex.com

→ Licensing
M. licensing@inscreenex.com

→ Intended Use and Licensing

This product is intended for in vitro laboratory research use only. It is not intended for any animal or human therapeutic or diagnostic use.

If you have purchased this cell line for **academic, non-profit research**, the use of this cell line is governed by the **in**screenex *Limited Research Use License (LRUL)*. Please refer to the LRUL for the full terms and conditions, and relevant use limitations. If you have purchased this cell line for **commercial, for-profit research**, the use of this cell line is governed by the respective license agreement. Please refer to the agreement for the full terms and conditions, and relevant use limitations. If you wish to use the cell line for commercial, for-profit purposes please contact **licensing@inscreenex.com**.

→ Background Information

CI-huOB is an immortalized human osteoblast cell line

Catalog number: *INS-CI-1005*
Biosafety level (BSL): *Level 1*
Organism: *Homo sapiens (human)*
Tissue: *femoral head*
Donor info: *female, 63, Caucasian*
Growth properties: *adherent*

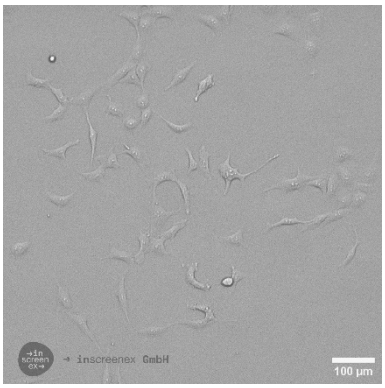
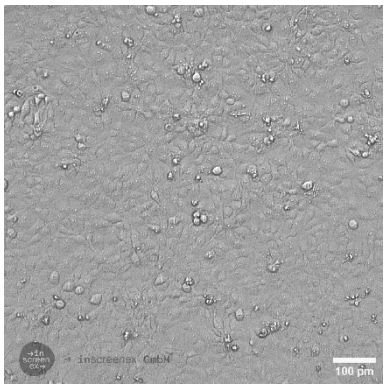
CI-huOB are immortalized human osteoblasts. Osteoblasts are specialized cells primarily responsible for bone formation and bone mineralization. They produce and secrete the organic components of the bone matrix, including collagen, which provides the framework for deposition of calcium and other minerals, the so-called mineralization.

CI-huOBs can be expanded in standard monolayer culture but undergo differentiation when cultured in our huOB Differentiation Medium (INS-ME-1007), where they deposit calcium and initiate mineralization. When combined with 3D cell culture conditions, CI-huOBs even form so-called mini-bones.

CI-huOBs were derived from primary cells isolated from the femoral head of the knee.

→ Morphology

adherent; grows in monolayer

Low confluency	***High confluency***
	

→ Cell Characterization and Assays

→ Marker Expression

The following cell-type specific markers were detected in CI-huOB:

Not available

→ Established Assays

CI-huOB were used in the following assays:

Not available

→ Quality Control

Basic information on quality control can be found below. For more details, request a Certificate of Analysis (CoA) by emailing **info@inscreenex.com** *and stating your Lot number.*

Cell number: *>0.5Mio viable cells*
Viability: *>75% post-thaw viability*
Sterility: *no contamination detected*
Mycoplasma: *no contamination detected*
Human pathogens: *primary material negative for HBV, HCV, HIV-1/HIV-2*
STR: *Not available*

→ Related Products

Products that are related to the CI-huOB cell line and are either required for a successful cell culture or recommended.

Required	***Recommended***
Medium: *huOB Maintenance Medium (INS-ME-1006)* *huOB Differentiation Medium (INS-ME-1007)*	***Cryopreservation:*** *Cell Freezing Medium (INS-SU-1027)*

→ Upon Arrival

Cells are routinely shipped on dry ice. Check all containers for leakage and breakage. Check if cells arrived frozen.

If, immediately upon arrival:

- *the vial appears damaged,*
- *the dry ice level in the shipping container appears low,*
- *the cells appear thawed, or*
- *you have any other concerns regarding the quality of the cells,*

do the following:

1) *take photos of the vial and/or the shipping container,*
2) *contact us by email or telephone (see* **General Inquiries** *on page 2**).*

If everything looks good, either seed the cryopreserved cells immediately, or store them:

- *at -80°C for periods of up to 2 days, or*
- *below -130°C in liquid nitrogen vapor, for long term storage.*

→ Medium Storage and Preparation

Our huOB Maintenance Medium is shipped as a kit that consists of:

- *30ml Supplements*
- *500ml Basal Medium*

Both components need to be combined according to the instructions below to obtain the complete, ready-to-use medium.

Our huOB Differentiation Medium is shipped as a kit that consists of:

- *10ml Supplements*
- *100ml Basal Medium*

Both components need to be combined according to the instructions below to obtain the complete, ready-to-use medium.

→ ***Cell culture antibiotics:*** *The medium does not contain prophylactic antibiotics. If you wish to use antibiotics, any standard, cell culture grade antibiotics can be added to the medium.*

Required materials	***Protocol***
– *30ml Supplements (10ml Diff. Med.)* – *500ml Basal Medium (100ml Diff. Med.)*	*1) Thaw Supplements at ambient temperature.* *2) Add 30ml Supplements to 500ml Basal Medium to obtain the complete Medium.* *To prepare smaller volumes of complete Medium, aliquot Supplements and store at -20°C. For example, aliquot 5×6ml and then add 6ml Supplements to 100ml Basal Medium.*
Storage	
– *Store* **Supplements** *at -20°C, if not indicated otherwise on the product label.* – *Store* **Basal Medium** *at 2-8°C, if not indicated otherwise on the product label.* – *Store* **complete Medium** *at 2-8°C. Complete Medium is stable for at least 1 month at 2-8°C.*	

→ Thaw Cryopreserved Cells

Do not thaw the cells until the recommended medium and coated flasks are on hand. For initial recovery (after delivery of the cells), we recommend thawing the cells on a T25 flask.

Required materials	***Protocol***
– *Cell culture vessel* – *Complete Medium, pre-warmed to 37°C (see section* **→ Medium Storage and** Preparation *on page 5)* – *15ml tube with a conical bottom suitable for centrifugation (e.g. "Falcon tube")*	*1) Add 4ml pre-warmed medium to a 15ml tube.* *2) Quickly thaw the cryovial at 37°C in a water bath until only a few ice crystals are visible. Disinfect vial briefly by spraying with 70% Ethanol.* *3) Transfer thawed cell suspension to the 15ml tube containing 4ml medium. Avoid excessively pipetting up and down.* *4) Centrifuge cells at 300×g for 5min.* *5) Aspirate supernatant.* *6) Gently resuspend the cell pellet in complete Medium. Use a volume appropriate for the cell culture vessel.* *7) Transfer cells in coated cell culture vessel and place in the incubator (37°C, 5% CO2).* *8) Change the medium after 2 days.*

→ Freeze Cells for Cryopreservation

Cell should be grown to 90% confluence before cryopreservation. Avoid full confluence before cryopreservation.

Required materials	***Protocol***
– *Cell Freezing medium (INS-SU-1027)* – *PBS* – *Trypsin/EDTA solution (TE)* – *2% FBS in PBS* – *15ml tube* – *Cryovial(s)* – *Freezing container ("Mr. Frosty" or similar)* – *15ml tube with a conical bottom suitable for centrifugation (e.g. "Falcon tube")*	*1) Aspirate medium.* *2) Wash with PBS and aspirate PBS.* *3) Add Trypsin/EDTA (TE) solution to the cells and incubate at room temperature or 37°C for 5-10min, or until the cells detach.* *4) Examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells.* *5) Resuspend cells in 2% FBS in PBS and transfer to a 15ml conical bottom tube.* *6) Centrifuge cells at 300×g for 5min.* *7) Aspirate supernatant and gently resuspend cell pellet in Freezing medium (approx. 1Mio. cells/ml).* *8) Transfer cell suspension into cryovial(s) and place them into a freezing container ("Mr.Frosty" or similar).* *9) Place the freezing container at-80°C for 16-24h.* *10) Transfer cryovials to liquid nitrogen vapor for long-term-storage. Add 4ml pre-warmed medium to a 15ml tube.*

→ Routine Cell Culture

Work in a sterile environment and follow Good Cell and Tissue Culture Practice.

Temperature: *37°C*
Environment: *5% CO_2 (v/v), humidified atmosphere*
Split ratio: *1:2 for initial split after thawing, 1:5 to 1:10 for routine culture*
Confluence: *split at 70-90% confluence*
Medium change: *every 2-3 days*

Required materials	***Protocol***
– *Cell culture vessel* – *Complete Medium, pre-warmed to 37°C (see section* → **Medium Storage and** Preparation *on page 5)* – *PBS* – *Trypsin/EDTA solution (TE)*	*1) Aspirate medium.* *2) Wash with PBS and aspirate PBS.* *3) Add Trypsin/EDTA (TE) solution to the cells and incubate at room temperature or 37°C for 5-10min, or until the cells detach.* *4) Examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells.* *5) Resuspend cells in complete Medium thereby inactivating the Trypsin/EDTA (TE) solution.* *6) Transfer an aliquot of the cell suspension to a new cell culture vessel containing fresh complete Medium.* *7) Place into incubator.*

Recommended volumes

Flask or Plate	***Medium or PBS***	***TE solution***
T75	*8-10ml*	*3ml*
T25	*4-5ml*	*1ml*
6-well	*1.5-3ml*	*0.7ml*
12-well	*1-2ml*	*0.25ml*
24-well	*0.5-1ml*	*0.1ml*
48-well	*0.2-0.4ml*	*75µl*
96-well	*0.1-0.2ml*	*50µl*

→ References

Lipps, Christoph et al. “Expansion of functional personalized cells with specific transgene combinations.” Nature communications vol. 9,1 994. 8 Mar. 2018, doi:10.1038/s41467-018-03408-4