CI-huOB

Cat. No.: INS-CI-1005

Biosafety Level	Storage	Culture Conditions
Level 1	vapor phase of liquid nitrogen	huOB Maintenance Medium (INS-ME-1006) huOB Differentiation Medium (INS-ME-1007)

General Information

CI-huOB is a human osteoblast cell line that was immortalized using the CI-SCREEN technology (Lipps et al. 2018; *Nat Comm*). The cell line shows alkaline phosphatase activity and calcium deposit.

Organism: Homo sapiens (human)
Tissue: femoral head
Growth properties: adherent

Cell culture media and reagents

Product	Cat. No.	Volume
huOB Maintenance Medium (includes basal medium and supplements)	INS-ME-1006	500ml
huOB Differentiation Medium (includes basal medium and supplements)	INS-ME-1007	100ml
Freezing medium	INS-SU-1004	30ml

Note: *The medium does not contain antibiotics. However, it may simply be supplemented with standard antibiotics.*

Intended Use

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

Quality control

Each vial contains ≥5×10^5 cells. Viability is ≥80%. Cells are negative for mycoplasma contamination. The source material is tested negative for HIV, HBV and HCV.

Upon arrival

Cells are routinely shipped on dry ice. Check all containers for leakage and breakage. Check if cells arrived frozen. After arrival, store the cryopreserved cells in liquid nitrogen vapor, or seed them immediately (please see page 2).

Note: *Cells may be stored at -80°C for short periods (<2 days), but this results in reduced viability and irreversible cell damage.*

Medium storage and preparation

Medium including supplements is shipped cooled at 4-8°C. Store reagents according to the instructions below upon arrival.

Material:

- huOB Maintenance Medium (INS-ME-1006). Includes Basal Medium (500ml) and Supplements (30ml; INS-ME-1006BS).
- huOB Differentiation Medium (INS-ME-1007). Includes Basal Medium (100ml) and Supplements (12ml; INS-ME-1007BS).

Storage:

- Store Basal Medium at 4-8°C
- Store Supplements at -20°C
- Store completed medium (Basal Medium plus Supplements) at 4-8°C. Completed medium is stable for at least 1 month at 4-8°C.

Protocol:

1) Thaw Supplements at 15-25°C.
2) Add 30ml Supplements to 500ml Basal Medium and store at 4-8°C. For the Differentiation Medium, add 12ml Supplements (INS-ME-1007BS) to 100ml Basal Medium (INS-ME-1007). Completed medium is stable for at least 1 month at 4-8°C.

Note: *Supplements may be aliquoted and stored at -20°C before completing the medium. For example, for Maintenance Medium, aliquot 5×6ml and then add 6ml Supplements to 100ml Basal Medium.*

Recover cryopreserved cells

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

Material:

- cell culture vessel(s)
- complete medium
- 15ml tube

Protocol:

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 with 70% Ethanol.
3) Transfer thawed cell suspension to the 15ml tube containing 4ml medium. Avoid excessive pipetting up and down.
4) Centrifuge cells at 200×*g* for 4min.
5) Aspirate supernatant.
6) Gently resuspend the cell pellet in complete medium.
7) Transfer cells in coated cell culture vessel and place in the incubator (37°C, 5% CO_2).
8) Change the medium after 2 days.

Routine Subculture

Change medium every 2-3 days and split the cells at 70-90% confluence. The split ratio after recovery from cryopreservation should not exceed 1:2. For routine maintenance, split ratio can be increased to 1:5 to 1:10.

Material:

- cell culture vessel(s)
- complete medium
- PBS
- Trypsin/EDTA solution (TE)

Protocol:

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 attach.
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 coated cell culture vessel containing fresh complete medium.
7) Incubate at 37°C and 5% CO_2.

Vessel	Medium or PBS (ml)	TE (ml)
T75 flask	8-10	2
T25 flask	4-5	1
6well	1.5-2	0.5
12well	1	0.2
24 well	0.5	0.1
96well	0.1	0.05

Cryopreservation

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

Material:

- Freezing medium (INS-SU-1004)
- PBS
- Trypsin/EDTA solution (TE)
- 2% FBS in PBS
- 15ml tube
- cryovial(s)
- freezing container (“Mr. Frosty” or similar)

Protocol:

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 attach.
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 tube.
6) Centrifuge cells at 200×*g* for 4min.
7) Aspirate supernatant and gently resuspend cell pellet in Freezing medium (approx. 1×10^6 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 -70 to -80°C for 16-24h.
10) Transfer cryovials to liquid nitrogen vapor for long-term-storage.

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