Human Osteoclast Culture Kit

For the culture of human osteoclasts from precursor cells.

Cat. No.: KT-791

For Research Use Only.
PRODUCT INFORMATION
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PRINCIPLE
In aging societies, osteoporosis and other age-related bone metabolism disorders are a rapidly increasing problem. The amount of bone in an organism is controlled by a balance of osteoblasts (bone-forming cell) and osteoclasts (bone-destroying cell) activities. A method to induce osteoclasts formation from bone marrow cells using M-CSF (macrophage-colony stimulating factor) and RANKL (receptor activator of NF-κB ligand) has been established in recent years.

This kit includes cryopreserved primary precursor osteoclasts from human bone marrow and media formulated for the optimal culture of human osteoclast in vitro. These are sterile, liquid basal media (α-MEM) which contain essential and non-essential amino acids, vitamins, other organic compounds, trace minerals, inorganic salts, growth factors, hormones, fetal bovine serum, and antibiotics. The culture medium, which is used to differentiate preosteoclasts into mature osteoclasts, contains 50 ng/mL M-CSF and 100 ng/mL RANKL.

COMPONENTS

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<tr>
<th>Human Osteoclast Culture Kit</th>
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<tbody>
<tr>
<td>Components</td>
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<tr>
<td>Human Osteoclast Precursor Cells, frozen</td>
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<tr>
<td>Washing Medium</td>
</tr>
<tr>
<td>Culture Medium, with M-CSF (50 ng/mL) and RANK Ligand (100 ng/mL)</td>
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Storage

<table>
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<tr>
<th>Components</th>
<th>Storage Conditions</th>
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<tbody>
<tr>
<td>Human Osteoclast Precursor Cells</td>
<td>Liquid Nitrogen (preferred)</td>
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<tr>
<td>Washing Medium</td>
<td>-20°C Freezer</td>
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<tr>
<td>Culture Medium</td>
<td>-20°C Freezer</td>
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Materials required but not provided
- Pipettes
- 96-well, flat bottom culture plate
- Tubes
- Refrigerated centrifuge
- Water bath

PRECAUTIONS
1. Read the instructions carefully before beginning the culture.
2. This kit is for research use only, not for human or diagnostic use.

PROTOCOL
1. Thaw the Wash and Culture Medium in a 37°C water bath with gentle shaking.
2. Quickly thaw the osteoclast precursors in a 37°C water bath.
3. After thawing, transfer the cells to a 15 mL centrifuge tube containing 10 mL of Wash Medium and mix gently. Centrifuge 200 x g for 5 minutes at 4°C.
4. Remove supernatant and suspend the cells in 10 mL of Wash Medium. Centrifuge at 200 x g for 5 minutes at 4°C.
5. Remove supernatant and resuspend the cells in 2.5 - 5 mL of Culture Medium containing M-CSF and RANKL.
6. Transfer 100 µL of cell suspension into each well of a 96-well plate. If the cells are resuspended in 5 mL of Culture Medium, there will be enough cell suspension for about 50 wells. To quickly observe osteoclasts formation, culture the cells at a higher density.
7. Incubate the plates at 37°C, 5% CO₂, 100% humidity.
8. Precursor cells may be sticky and form clumps of cells containing cell debris. DO NOT throw the clumps out as they contain viable cells. Replace Culture Medium within 3-4 days. If the first medium change is later than day 3 or 4, fewer osteoclasts may develop.
9. After adding fresh medium on day 3 or 4, change the medium every other day. Cells will begin to fuse and form osteoclasts around day 5 (Fig 1). Feeding the cells with fresh medium on a frequent basis will maintain the osteoclasts.
10. To visualize osteogenesis, count the osteoclasts by staining with tartrate-resistant acid phosphatase (TRAP staining, Fig 2, Catalog # KT-008).

![Figure 1: Phase contrast microscopy of differentiated osteoclasts](image)

**EXAMPLES AND APPLICATIONS**

1. TRAP Staining Kit (Cat. No. KT-008)
   Osteoclasts were fixed then stained with 5 mL of a mixture containing chromogenic substrate and tartrate-containing buffer.

![Figure 2: TRAP stained osteoclasts](image)

2. TRAP analysis of culture supernatant is qualitative (Catalog # KT-008)
   Thirty microliters of culture supernatant was incubated for 3 hours in the presence of chromogenic substrate/tartrate-containing buffer. The samples were read at wavelength 540 nm.
3. Pit Assay
Primary precursor osteoclasts cultured on ivory for 7 – 14 days. The section was sonicated in 5 mL of 1M ammonia solution to disrupt the cells. The ivory section was stained with Mayer’s hematoxylin solution for 1 minute then washed and dried.

4. Scanning electron microscopy (SEM):
SEM of the ivory section used in the Pit assay.