

Poietics™ human osteoclast precursors

Instructions for use

Product application: For *in vitro* assays of human osteoclast differentiation and function.

Receiving instructions: Unpack immediately! Packages may contain components with various storage requirements!

Safety statements

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use *in vitro* diagnostic or clinical procedures.

WARNING: CLONETICS™ AND POIETICS™ PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA approved method for the presence of HIV-1, hepatitis B virus and hepatitis C virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, hepatitis B virus, and hepatitis C virus. Testing cannot offer complete assurance that HIV-1, hepatitis B virus, and hepatitis C virus are absent. All human sourced products should be handled at the biological safety level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH manual, [Biosafety in Microbiological and Biomedical Laboratories](#), 5th edition. If you require further information, please contact your site safety officer or scientific support.

Protocol details

Unpacking and storage instructions

1. Check all containers for leakage or breakage.
2. For cryopreserved cells – remove cryovials from the dry ice packaging and immediately place into liquid nitrogen storage. Alternatively, thaw and use the cells immediately. If no dry ice remains, please contact customer service.
3. BulletKit™ instructions: Upon arrival, store basal medium at 4-8°C and SingleQuots™ at –20°C in a freezer that is not self-defrosting. Using media or reagents other than what's recommended will void the cell warranty. Please contact scientific support if you need help selecting media and/or reagents.

Cell isolation/cryopreservation

1. Primary human osteoclast precursors are isolated by density gradient centrifugation, immunoaffinity purification and selective culturing techniques.
2. Primary human osteoclast precursors are cryopreserved in a solution containing 86.5%

IMDM, 7.5% DMSO, 4% human serum albumin and 2% hydroxy-ethyl-starch.

Preparation of media

1. Use pre-warmed (37°C), supplemented medium for culturing osteoclast precursors.
2. Decontaminate the external surfaces of a 100 ml bottle of osteoclast precursor basal medium with 70% v/v ethanol or isopropanol.
3. Make up osteoclast precursor growth medium by adding the FBS, L-glutamine, penicillin and streptomycin SingleQuots™ to the bottle of osteoclast precursor basal medium (the final concentrations of the supplements once the SingleQuots™ are added will be 10%, 2 mM, 100 units/ml and 100 µg/ml respectively).

Thawing of cells / initiation of culture process

NOTE: The thawing procedure described below has been developed to provide optimal recovery and cell viability. Failure to follow this protocol will result in lower yields of viable progenitor cells.

1. Warm 100 ml of osteoclast precursor growth medium in a 37°C water bath.
2. Quickly but completely, thaw the vial of frozen cells in a 37°C water bath, about 1-2 minutes. Wipe the outside of the vial with 70% ethanol.
3. Aseptically transfer the cell suspension to a 50 ml conical tube.
4. Rinse the cryovial with 1 ml of osteoclast precursor growth medium. Add the rinse dropwise to the cells while gently swirling the tube (~1 minute).
5. Slowly add additional medium drop-wise to the cells until the total volume is 5 ml, while gently swirling after each addition of several drops of medium (~3 minutes).
6. Slowly bring the volume up to 40 ml by adding 1 to 2 ml volumes of medium drop wise, while gently swirling after each addition of medium (~10 minutes).
7. Centrifuge the cell suspension at 200 x g at room temperature for 15 minutes.
8. Carefully remove by pipette and save most of the wash, leaving approximately 3 ml behind so the

cell pellet is not disturbed. Gently resuspend the cell pellet in the remaining medium and transfer to a 15 ml conical tube.

9. Rinse the 50 ml conical tube with 2 ml of osteoclast precursor growth medium and add drop-wise to the cells in the 15 ml conical tube.
10. Slowly bring the volume up to 10 ml by adding 1 to 2 ml volumes of osteoclast precursor growth medium drop-wise while gently swirling after each addition of medium.
11. Centrifuge the cell suspension at 200 x g at room temperature for 15 minutes.
12. Carefully remove by pipette all but 1 ml of the wash. Gently resuspend the cell pellet in the remaining medium and count. When washing the cells, do not attempt to remove too much of the wash. Leave a minimum of 1 ml of wash at the bottom of the tube. If the final cell count is low, some of the pellet may have been removed with the wash.
13. Dilute 20 µl of the cell suspension in 20 µl of 0.4% trypan blue and do a cell count and determine % viability. Recovery should be greater than 90%. If the cell count is lower than expected, centrifuge the wash saved in step # 8 at a higher speed, count and combine if necessary.

Maintenance

Primary human osteoclast precursors cannot be “passaged”. They can be differentiated, but in the absence of specific differentiation signals, the cells will senesce.

Osteoclast precursor differentiation procedure

Prepare osteoclast differentiation medium

If the precursors are not to be treated with test samples:

1. We suggest that, as a negative control, some wells be cultured in the absence of soluble RANK ligand. While the precursors will expand in number, no functional differentiated osteoclasts will develop in these controls.
2. Add the entire contents of the M-CSF SingleQuots™ to **30 ml** of osteoclast precursor growth medium - the final concentration will be 33 ng/ml.

Note: The vial of M-CSF may have to be centrifuged at very low speed to recover the entire content of the vial. Remove 1 ml of the M-CSF supplemented medium for the culture of undifferentiated control cells.
3. Add 1.0 ml of the medium containing M-CSF to the vial of lyophilized soluble RANK ligand. Cap the

vial, mix and remove the contents and add the RANK ligand SingleQuots™ to the remaining 28 ml of the M-CSF supplemented medium. The final concentration of soluble RANK ligand will be 66 ng/ml.

4. Add osteoclast precursors to the control and differentiation medium at a concentration of **50,000 cells/ml**. Seed 10,000 osteoclast precursors/well at **0.2 ml/well**.

If the precursors are to be treated with test samples:

1. We suggest that, as a negative control, some wells be cultured in the absence of soluble RANK ligand. While the precursors will expand in number, no functional differentiated osteoclasts will develop in controls containing no soluble RANK ligand.

Add the entire contents of the M-CSF SingleQuots™ to **15 ml** of osteoclast precursor growth medium - the final concentration will be 33 ng/ml upon addition of 0.1 ml of test sample. Note: The vial of M-CSF may have to be centrifuged at very low speed to recover the entire content of the vial. Remove 0.5 ml of M-CSF supplemented medium for the culture of undifferentiated control cells.

Add 1.0 ml of the medium containing M-CSF to the vial of lyophilized soluble RANK ligand. Cap the vial, mix and remove the contents and add the RANK ligand SingleQuots™ to the remaining 13.5 ml of the M-CSF supplemented medium. The final concentration of soluble RANK ligand will be 66 ng/ml upon addition of 0.1 ml of test sample.

2. Add osteoclast precursors to the control and differentiation medium at a concentration of **100,000 cells/ml**. Seed 10,000 osteoclast precursors/well at **0.1 ml/well**.
3. Set up a 24-well dilution plate with appropriate volumes of osteoclast precursor growth medium/well and make serial dilutions of the test sample(s) to be assayed. Add 0.1 ml of each different concentration of test sample, to the wells of osteoclast precursors. Each assay should be done in triplicate.
4. We suggest that “control” wells be set up which contain 1) no added test sample and 2) solvent only” if the test samples were dissolved in solvents such as DMSO, ethanol, etc.

Cell culture

1. Incubate the cells at 37°C, in a humidified atmosphere of 5% CO₂.
2. Day 7 osteoclasts can be identified by phase microscopy as unusually large multinucleate cells. The majority of each well’s bottom surface should be covered by such cells. The culture can be

continued for an additional week, with or without feeding, during which time the osteoclasts will continue to increase in size.

- To document osteoclast differentiation, cultures may be stained for the $\alpha_v\beta_3$ integrin complex or for tartrate-resistant acid phosphatase.

Ordering information

Cryopreserved cells

2T-110 OCP ≥ 1 million cells

Note: In order to culture and differentiate primary human osteoclasts into functional osteoclasts, the following cell culture medium, supplements and cytokines will be required.

Related products

Osteoclast growth media (must be purchased separately)

PT-8001	OCP BulletKit™	Includes basal medium and SingleQuots™ for growth and differentiation of primary human osteoclast progenitors
PT-8201	OCP basal medium	Osteoclast precursor basal medium (100 ml)
PT-9501	OCP Growth medium SingleQuots™ kit	Supplements and growth factors (FBS, L-glutamine, penicillin/streptomycin, M-CSF and soluble RANK ligand)
PA-1000	OsteoAssay™ human bone plate	96-well plate format
PA-4490	CalciFluor™ assay	Calcium detection reagent, diluent, basal resorption medium, calcium standard and instructions

Product warranty

CULTURES HAVE A FINITE LIFESPAN *IN VITRO*. Lonza warrants its cells only if Poietics™ media are used, and the recommended protocols are followed. Cryopreserved human osteoclast precursors are assured to be viable and functional when thawed and maintained properly.

Quality control

For detailed information concerning QC testing, please refer to the certificate of analysis.

References

- Roodman, GD. Cell biology of the osteoclast. *Exp Hematol.* 1999 Aug;27(8):1229-41.
- Nesbitt S, Nesbit A, Helfrich M, Horton M. Biochemical characterization of human osteoclast integrins. Osteoclasts express $\alpha_v\beta_3$, $\alpha_2\beta_1$, and $\alpha_v\beta_1$ integrins. *J Biol Chem* 1993 Aug 5;268(22):16737-45.
- Janckila AJ, Li CY, Lam KW, Yam LT. The cytochemistry of tartrate-resistant acid phosphatase. Technical considerations. *Am J Clin Pathol* 1978 Jul;70 (1):45-55.
- Alatalo SL, Halleen JM, Hentunen TA, Monkkonen J, Vaananen HK. Rapid screening method for osteoclast differentiation in vitro that measures tartrate-resistant acid phosphatase 5b activity secreted into the culture medium. *Clin Chem* 2000 Nov;46(11):1751-4.