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OsteoAssay™ Human Bone Plate

Instructions for Use

Product Application: The OsteoAssay plate provides a matrix for the culture of osteoclasts and the assay of osteoclast bone resorption activity.

Receiving Instructions: Store at -20°C in a dry environment.

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 clinical or in vitro 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-I, 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 can not 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, 1999. If you require further information, please contact your site Safety Officer or Technical Services.

Product Description

The OsteoAssay plate provides a thin layer of adherent human bone particles for the culture of primary human or non-human osteoclasts[1], osteoclast precursors and osteoclast cell lines. Cells can be seeded onto the surface of the OsteoAssay plate in a manner identical to that used in traditional cell culture protocols. Cells can be stained with standard cytochemical (e.g. TRAP[2]) or immunofluorescent techniques[3]. The production of bone resorption-related products (e.g. collagen peptides[4]) and/or enzyme activity[5] can be measured by sampling the cell culture supernatant after an appropriate period of cell culture.

Use of the OsteoAssay Plate for the Culture of Osteoclasts

In a laminar flow cell culture hood, remove the OsteoAssay plate from its package and let it come to room temperature (60 minutes before use) – do not warm the plate above room temperature at this point.

If the entire OsteoAssay plate is not to be used in a single assay, unused strips of OsteoAssay wells can be placed in the frame of a Corning Costar strip well plate (Costar # 9012 – not provided) and stored in the original foil zip lock OsteoAssay plate package at –20°C.

Remove strips by applying pressure to the center bottom (underside) of each well in a strip until the entire strip loosens. The entire strip can then be removed by grasping the protrusions at each end of the strip. Failure to loosen the strip before attempting its removal may break the plastic strip. To maintain sterility, be careful not to touch the top surface of the wells.

Each OsteoAssay strip removed should be replaced with plain strip wells filled with 200 µl/well of water to prevent excess evaporation of cell culture medium.

If using primary human osteoclast precursors (Lonza product # 2T-110), seed each well of the OsteoAssay plate with 10,000 precursors in the recommended medium and growth supplements (Lonza product PT-8001). Other cells types can be used and other media formulations may work, but protocols for cells and media other than those described above will have to be optimized and validated by the user.

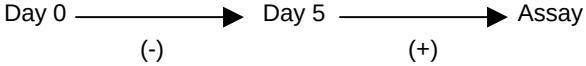
If using primary human osteoclast precursors, renew the medium after 5 to 7 days. New media must contain the appropriate concentrations of M-CSF and soluble RANK ligand. Unused media made on day 0 can be frozen and used for the day 5 medium change. Aspiration of the medium must be done carefully so as to avoid removal of the bone particles themselves.

Use of the OsteoAssay Plate for the Assay of Osteoclast Function

The OsteoAssay plate provides a means of assaying bone resorption using normal human tissue. If using primary human osteoclast precursors, allow a minimum of 5 days of culture for the differentiation of the cells. Supernatants can be assayed for the products of in vitro bone degradation and/or osteoclast differentiation. A variety of commercially available EIA kits are available for such purposes (e.g. The Quidel Metra Helical Peptide EIA kit).

For assays of in vitro bone resorption:

To test the effect of different agents on bone resorption by differentiated osteoclasts, add the agent to be tested with the new medium on day 5-7. The supernatants can then be harvested for assays after 1 to 4 days of additional culture. In the diagram below, (+) denotes the presence of the test sample in the culture medium; (-) denotes absence of test sample.



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For assays of Precursor differentiation:

If the OsteoAssay plate is to be used to assay for inhibition of precursor differentiation, test samples should be added at day 0 and removed with the day 5-7 media change.

Day 0 ⟶ Day 5 ⟶ Assay

(+) (-)

Figure 1 documents the superiority of the OsteoAssay matrix relative to dentine slices.

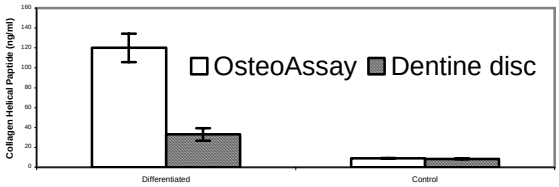

Figure 1. Comparison of primary human osteoclast function (in vitro bone degradation) when cultured on an OsteoAssay plate vs. dentine slices. Primary human osteoclast precursors were seeded at 10,000 cells/well in the presence of either M-CSF alone (control/undifferentiated) or both M-CSF and soluble RANK ligand (differentiated) for 5 days. Media were renewed after 5 days and supernatants were harvested after an additional 1 day of culture and assayed for collagen peptides (helical peptide EIA kit).

Ordering Information

OsteoAssay Human Bone Plate	
PA-1000	96-well stripwell plate

Related Products

Osteoclast Cell System (Must be purchased separately)

2T-110	Osteoclast Precursors (OCP)	>1 million cells/cryovial
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 SingleQuot Kit	Supplements and growth factors (FBS, L-glutamine, Penicillin/ Streptomycin, M-CSF and Soluble RANK ligand)

Product Warranty

Lonza warrants the OsteoAssay plate, to the original purchaser only, against defects in materials and workmanship under use and application as described in the Instruction Manual. OsteoAssay plate products are not for resale. Commercialization of product using components of the OsteoAssay plate requires an express license under applicable patents and intellectual property from Lonza Walkersville, Inc.

Quality Control

For detailed information concerning QC testing, please refer to the Certificate of Analysis.

References

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2. 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.
3. Nesbitt S, Nesbit A, Helfrich M, Horton M. Biochemical characterization of human osteoclast integrins. Osteoclasts express alphavbeta3, alpha2beta1, and alphavbeta1 integrins. J Biol Chem 1993 Aug 5;268(22):16737-45.
4. Stroup GB, Lark MW, Veber DF, Bhattacharyya A, Blake S, Dare LC, Erhard KF, Hoffman SJ, James IE, Marquis RW, Ru Y, Vasko-Moser JA, Smith BR, Tomaszek T, Gowen M. Potent and selective inhibition of human cathepsin K leads to inhibition of bone resorption in vivo in a nonhuman primate. J Bone Miner Res 2001 16:1739-1746.
5. 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.