Lonza Walkersville, Inc.
www.lonza.com
scientific.support@lonza.com
Scientific Support: 800-521-0390
Document # INST-AA-1048-8 04/11
Walkersville, MD 21793-0127 USA

OsteoLyse™ Assay Kit (Human Collagen)

Instructions for Use

Product Application: The OsteoLyse™ Assay Kit (Human Collagen) provides for the quantitative measurement of *in vitro* osteoclast-mediated degradation of human bone collagen (type I).

Receiving Instructions: Upon receipt, store at 4°C.

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. Materials tested and found negative for the presence of viral DNA from HIV-I, HIV-2 and HBV. All human based products should be handled at the Biological Safety Level 2 (or higher) 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

Product Description

The OsteoLyse™ Assay (Human Collagen) provides a 96-well OsteoLyse™ Cell Culture Plate coated with fluorophore-derivatized human bone matrix (europium-conjugated collagen) for use in assays of osteoclast differentiation and function. The assay is a direct measure of the release of matrix metalloproteinases into the resorption lacuna of the osteoclast[1]. Cells can be seeded onto the surface of the OsteoLyse™ Plate in a manner identical to that used in traditional cell culture protocols. The resorptive activity of the osteoclasts, as reflected by the release of Eu-labeled collagen fragments, can be measured by simply sampling the cell culture supernatant after an appropriate period of cell culture. The cell culture supernatants are added to Fluorophore-Releasing Reagent in a second 96-well assay plate and counted using time-resolved fluorescence[2].

OsteoLyse Assay Protocol

1. Remove the OsteoLyse™ Plate from 4°C storage and let it warm to room temperature.
2. Seed mature osteoclasts or osteoclast precursors (human or non-human) onto the OsteoLyse™ Cell Culture Plate in medium containing M-CSF and soluble RANK ligand. If using Lonza's Primary Human Osteoclast Precursors, seed the cells at a density of 10,000 cells/well in Osteoclast Precursor Differentiation Medium (product # PT-8001). See the Instructions for Use for a detailed protocol for the culture of Primary Human Osteoclast Precursors (product # 2T-110).
3. Precursors cultured in the absence of soluble RANK ligand can serve as "undifferentiated" controls.
4. Culture the cells for 5 to 7 days. Culture systems, which utilize cells, culture medium or cytokines other than those in Lonza's products, will require optimization.
5. Renew the cell culture media after 6 days. Note that the fresh medium must contain the same concentrations of M-CSF and soluble RANK ligand as in the original day 0 medium. Unused control and differentiation media from day 0 can be frozen (on day 0) and used for the day 6 medium changes.
6. If the OsteoLyse™ Assay is to be used to screen or otherwise assay test samples, there are two different protocols that may be used. If the assay is used to measure differentiation of the osteoclast precursor, the test sample should be added at day 0 and removed at day 6. However, if the assay is to measure mature osteoclast function (i.e. bone matrix collagen degradation), the test sample should be added only at day 6 with the new medium addition.
7. The cell culture supernatant can be sampled at any time after the medium change. Because a very small volume (5 to 10 µl) of supernatant is sampled, it is very easy to do time-course studies by sampling the supernatant on sequential days. Note that supernatant volumes greater than 10 µl are unnecessary and may lead to inefficient counting of the fluorophore as the ratio of

Fluorophore Releasing Reagent to sample decreases.

8. Prior to sampling the cell culture supernatant, remove the Fluorophore Releasing Reagent from 4°C storage and let it warm to room temperature – do not warm this reagent in a water bath.
9. Place 200 µl of Fluorophore Releasing Reagent in each well of the black 96-well assay plate (included in the OsteoLyse™ Assay Kit).
10. Transfer 10 µl of cell culture supernatant to the wells of the assay plate containing Fluorophore Releasing Reagent. Note that it is essential to change pipette tips each time a new cell culture supernatant is sampled.
11. Note that the europium fluorophore is extremely sensitive. All pipette tips and other materials that come into contact with the probe must be discarded in appropriate waste containers. Probe that is spilled on a counter top and allowed to dry will give rise to dust and high background levels in the laboratory. Fluorimeters that are contaminated with the probe must be cleaned. A good rule of thumb is to use the probe as one would use a radioactively labeled material.
12. Briefly mix the samples in the assay plate.
13. Determine the fluorescence of each well of the assay plate in a time-resolved fluorescence fluorimeter (e.g. a Wallac Victor, with excitation at 340 nm and emission at 615 nm) over a 400 µsecond time period after an initial delay of 400 µseconds.
14. If the amount of collagen degraded, as a percentage of the total available collagen, is to be calculated, determine the total amount of intact collagen/well by placing 200 µl of Fluorophore Releasing Reagent in each of three unused wells of the OsteoLyse™ Plate. Mix the contents of the wells and then transfer 1 µl/well to corresponding wells in an assay plate containing 200 µl of Fluorophore Releasing Reagent/well. Determine the fluorescence of each well of the assay plate in a time-resolved fluorescence fluorimeter and multiply the result by 200 to calculate the total amount of intact collagen/well.

Expected Results

The fluorescence of the supernatant samples diluted in the wells of Fluorophore Releasing Reagent is directly proportional to the resorptive activity of the mature osteoclast. The fluorescent read-out of the OsteoLyse™ Assay is proportional to cell number and the degree of osteoclast differentiation. See Figure 1 for documentation that the accumulation of collagen fragments is directly related to cell culture time.

Data from the OsteoLyse™ Assay correlate very well with more traditional assays such as the TRAP assay[3], although the OsteoLyse™ Assay is much more readily quantifiable and has a greatly increased throughput. Figure 2 demonstrates the inhibition of osteoclast differentiation by interferon γ[4], as measured with both TRAP staining and the OsteoLyse™ Assay. Figure 3 demonstrates that the OsteoLyse™ Assay can be used to assay the resorptive function of mature osteoclasts. The ability of the bisphosphonate alendronate to inhibit resorptive activity was assayed and an IC50 of approximately 3 µM was obtained, similar to that found in the literature[5]. A parallel TRAP assay demonstrated that low concentrations of alendronate, did not inhibit osteoclast precursor differentiation, in agreement with previously published data[5].

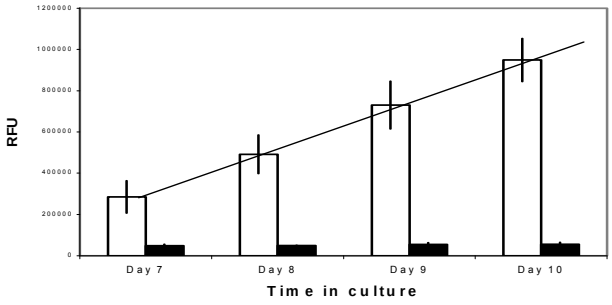

Figure 1. Primary Human Osteoclast Precursors were seeded onto an OsteoLyse™ Plate at 10,000 cells/well and differentiated with M-CSF and soluble RANK ligand. At days 7, 8, 9 and 10 of culture, 10 µl of supernatant was removed and counted. The black bars represent counts obtained when the precursors were cultured with M-CSF only.

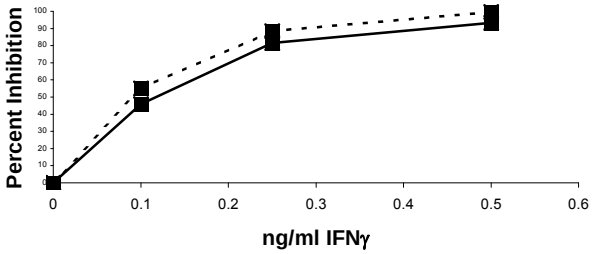

Figure 2. Primary human osteoclast precursors were seeded onto an OsteoLyse™ Plate at 10,000 cells/well and differentiated with M-CSF and soluble RANK ligand in the presence of various concentrations of interferon γ. At day 7 of culture, the cell culture medium was renewed. After an additional 24 hours of culture, 10 µl samples of supernatant were removed and counted. The dotted line denotes TRAP data (day 8 multinucleated TRAP-positive cells/well) while the solid line represents OsteoLyse™ Data.

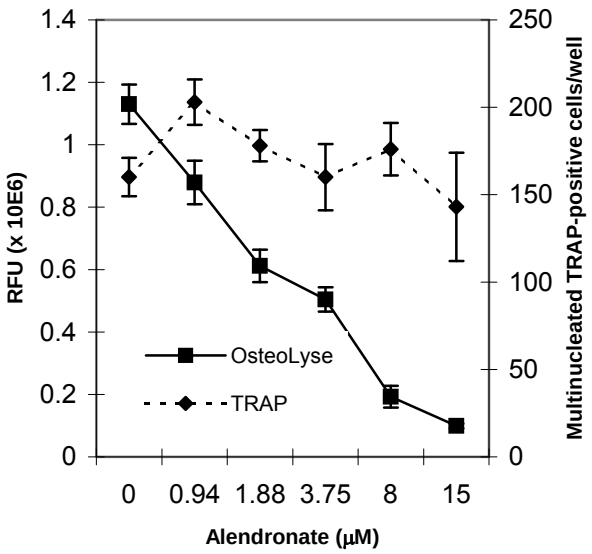

Figure 3. Primary Human Osteoclast Precursors were seeded onto an OsteoLyse™ Plate at 10,000 cells/well and differentiated with M-CSF and soluble RANK ligand in the presence of various concentrations of alendronate. At day 10 of culture, 10 µl samples of supernatant were removed and counted. The dotted line denotes TRAP data (day 10 multinucleated TRAP-positive cells/well) while the solid line represents OsteoLyse™ Assay Data.

Ordering Information

OsteoLyse™ Assay Kit (Human Collagen)		
PA-1500	Assay Kit	96-well OsteoLyse™ Plate, Fluorophore-releasing reagent, 96-well black assay plate and instructions

Related Products

Osteoclast Cell System (Must be purchased separately)		
2T-110	Osteoclast Precursors (OCP)	≥1 million cells/cryovial
PT-8001	OCP BulletKit™	Includes OCP 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)

Lonza

Product Warranty

Lonza warrants the OsteoLyse™ Assay Kit (Human Collagen), to the original purchaser only, against defects in materials and workmanship under use and application as described in the Instruction Manual. OsteoLyse™ Products are not for resale. Commercialization of products using components of the OsteoLyse™ Assay Kit (Human Collagen) 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

1. Delaisse JM, Andersen TL, Engsig MT, Henriksen K, Troen T and Blavier L. Matrix metalloproteinases (MMP) and cathepsin K contribute differently to osteoclastic activities. Microsc Res Tech. (2003) 61:504-13.
2. Hemmila I, Dakubu S, Mukkala VM, Siitari H and Lovgren T. Europium as a label in time-resolved immunofluorometric assays. Anal. Biochem. (1984) 137:335-343.
3. Janckila AJ, Li CY, Lam KW, Yam LT. The cytochemistry of tartrate-resistant acid phosphatase. Technical considerations. Am J. Clin. Pathol. (1978) 70 (1):45-55.
4. Kamolmatyakul S, Chen W and Li YP. Interferon-gamma down-regulates gene expression of cathepsin K in osteoclasts and inhibits osteoclast formation. (2001) J Dent Res. 80:351-5.
5. Azuma Y, Sato H, Oue Y, Okabe K, Ohta T, Tsuchimoto M. and Kiyoki M. Alendronate distributed on bone surfaces inhibits osteoclastic bone resorption *in vitro* and in experimental hypercalcemia models. (1995) Bone. 16:235-45.

Limited Use License

This product is distributed and sold to the End-User pursuant to a license from PerkinElmer Life Sciences, Inc. for use of its DELFIA technology by the End-User for life science research applications in the field of *in vitro* cell based assays combining *in vitro* cell culture and ELISA, but not for diagnostic applications. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the licensed use without the express written authorization of PerkinElmer Life Sciences, Inc. is strictly prohibited.