COSMO BIO USA
Inspiration for Life Science

Bone Biology Research Reagents

BONE RESORPTION ANALYSIS TOOLS

Bone Resorption Assay Kit

The Bone Resorption Assay Kit is for the measurement of cellular bone resorption activity.

Bone resorption activity releases fluoresceinamine-labeled chondroitin sulfate from plate-bound calcium phosphate. The coated calcium phosphate is first bound to fluoresce-inamine-labeled chondroitin sulfate (FACS), which is released from the calcium phosphate layer into conditioned medium by osteoclastic resorption activity. Bone resorption activity is evaluated by simply measuring the fluorescence intensity of the conditioned medium. This assay provides a rapid evaluation system unlike that of the traditional pit assay.

Features

- Bone resorption is evaluated by measuring the fluorescence intensity of the medium.
- Measurable at the same wavelength as FITC.
- Microscopic observation of cell morphology is possible.
- Pit area analysis can be done after removing cells.
- Ready to use, sterile components.

Kit components

- Bone Resorption Assay Plate
- Bone Resorption Assay FACS
- Bone Resorption Assay Buffer

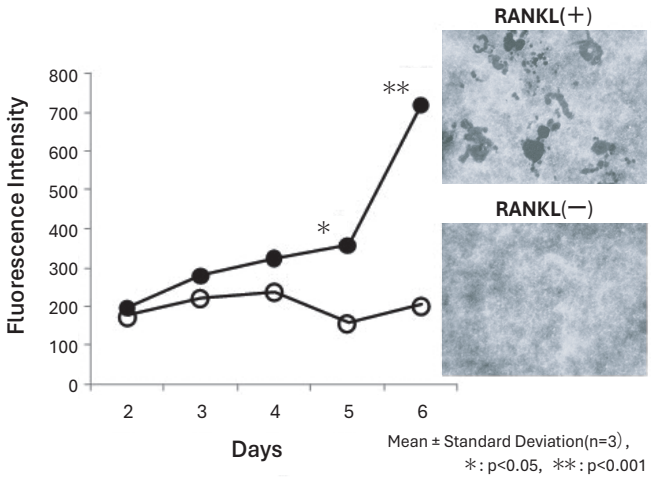


Fig 1. RANKL-mediated osteoclast differentiation. In the RAW264 macrophage cell line culture system, RANKL(-) shows no change in pit formation or fluorescence intensity in the culture supernatant. However, RANKL(+) induces pit formation and increased fluorescence intensity due to osteoclast differentiation.

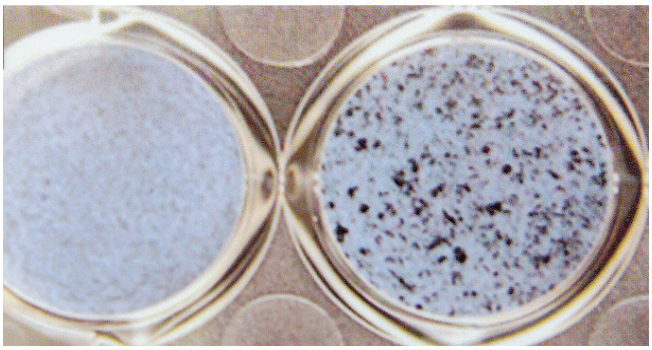


Fig. 2 Representative images of resorption pits after cell removal
Left: culture without RANKL
Right: culture with RANKL.
Pit formation is clearly observable by macroscopic inspection.

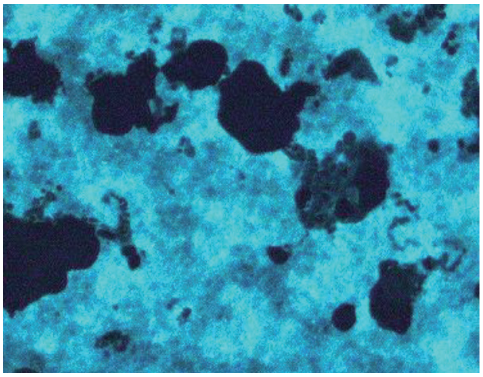


Fig. 3 Micrograph of the pits in a CaP-coated plate (with RANKL).

Pits formed under RANKL-supplemented conditions were photographed under a microscope using dark-field illumination. The black areas represent the formed pits.

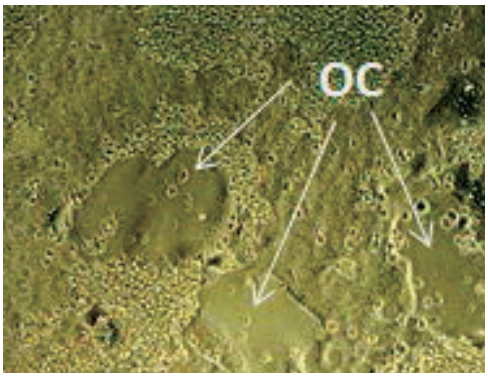


Fig. 4 Phase-contrast microscopy image of RAW264 cells.

RAW264 cells (day 6) cultured in the CaP-coated Bone Resorption Assay Plate stimulated with RANKL, 100 ng/mL. Osteoclast-like cells were observed.

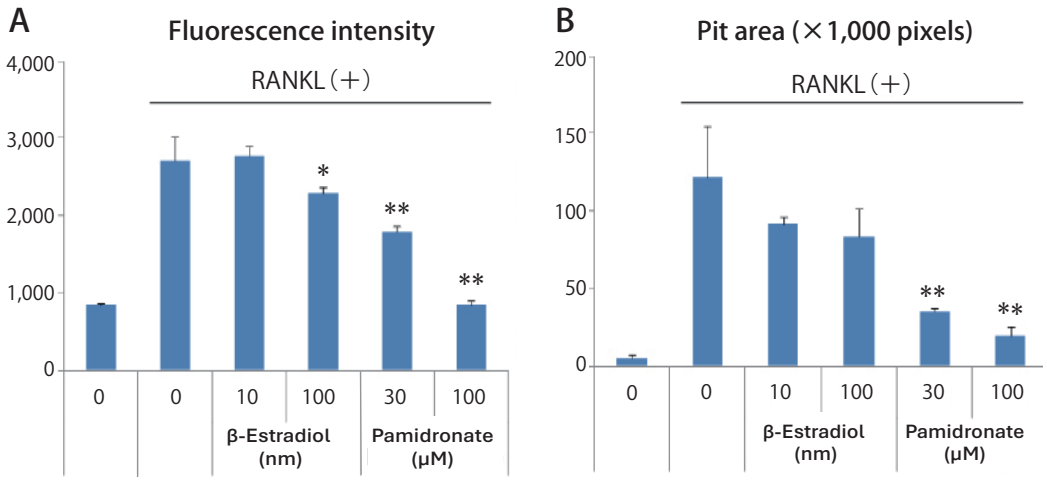


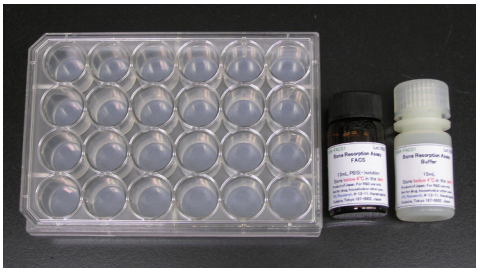
Fig. 5 Comparison of fluorescence intensity and pit area. In RAW264 cells, a RANKL concentration-dependent increase in fluorescence intensity (A) and an increase in pit area (B) were observed.

PRODUCT NAME	SIZE	CATALOG NUMBER
Bone Resorption Assay Kit	1 x 24 rxns	CSR-BRA-24KIT
	1 x 48 rxns	CSR-BRA-48KIT
	1 x 96 rxns	CSR-BRA-96KIT
	1 x 96 strip-well rxns	CSR-BRA-S96KIT
	2 x 24 rxns	CSR-BRA-24X2KIT
	2 x 48 rxns	CSR-BRA-48X2KIT
	2 x 96 rxns	CSR-BRA-96X2KIT
	2 x 96 strip-well rxns	CSR-BRA-S96X2KIT

Assay Plate for Bone Resorption Assay

Plates for Bone Resorption Assay

This is a culture plate coated with a solid layer of synthetic calcium phosphate (CaP) that is similar to natural apatite. It can be used as an alternative to dentin slices for evaluating bone resorption activity by cultured osteoclasts.



PRODUCT NAME	SIZE	CATALOG NUMBER
Bone Resorption Assay Plate	1 x 24 plate	CSR-BRA-24P
	1 x 48 plate	CSR-BRA-48P
	1 x 96 plate	CSR-BRA-96P
	1 x 96 strip-well plate	CSR-BRA-S96P
	2 x 24 plates	CSR-BRA-24X2P
	2 x 48 plates	CSR-BRA-48X2P
	2 x 96 plates	CSR-BRA-96X2P
	2 x 96 strip-well plates	CSR-BRA-S96X2P

Reagents for Bone Resorption Assay

PRODUCT NAME	SIZE	CATALOG NUMBER
Bone Resorption Assay Buffer	10 mL	CSR-BRA-B1
Bone Resorption Assay FACS	13 mL	CSR-BRA-FACS1

Octacalcium Phosphate Disk (OCP Disk)

Octacalcium phosphate (OCP) is a synthetic bone substitute material known for its excellent bone regeneration-promoting properties. This product is an OCP-based disk-shaped material designed for cell culture applications. It features a uniform composition and a smooth surface, making it suitable for bone metabolism research.

Bulk sizes are available.

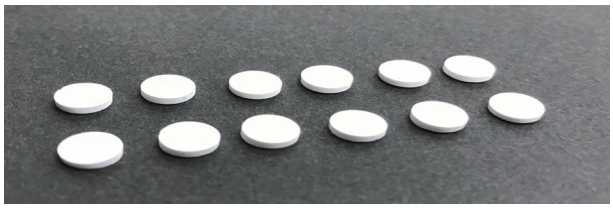


Fig. 1 Octacalcium Phosphate Discs.
Diameter 6 mm, Thickness 1 mm

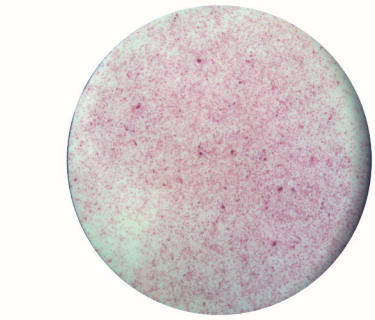


Fig. 2 TRAP staining of human osteoclasts seeded on OCP discs. Osteoclasts (Cat. No. PMC-OSC15C) were seeded onto OCP discs placed in a 96-well plate. After 7 days of culture using dedicated medium (Cat. No. PMC-OSCNW, PMC-OSCMHB), TRAP staining (Cat. No. PMC-AK04F) was performed.

PRODUCT NAME	SIZE	CATALOG NUMBER
Octacalcium Phosphate Disk (OCP Disk)	24 pcs	PMC-OCP-D24

Osteoclast Cells (Human, Rat, Mouse)

In the presence of M-CSF (Macrophage Colony Stimulating Factor) and RANKL (Receptor Activator of NFκB Ligand), osteoclast precursor cells will differentiat into osteoclasts.

For guaranteed optimal recovery and differentiation, we recommend Osteoclast Wash Medium (Cat. No. PMC-OSCMW) and CSF/RANKL-containing Osteoclast Culture Medium (for human) (Cat. No. PMC-OSCMHB). Together, these products are useful for studies of osteoclast formation, bone resorption, osteoporosis, rheumatoid arthritis, Paget's disease and others.

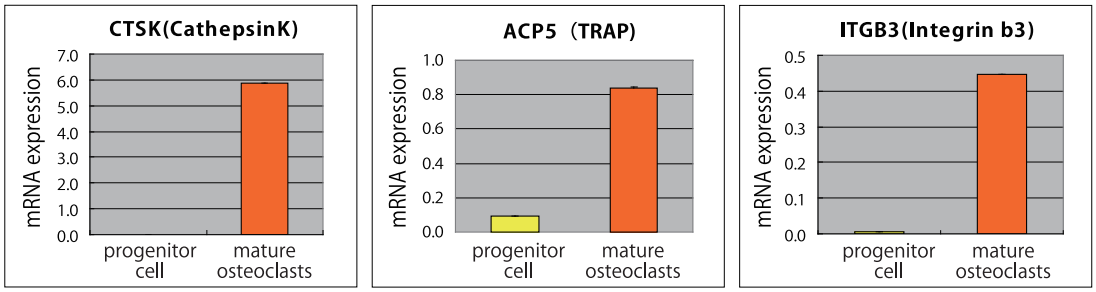


Fig. 1 Comparison of osteoclast marker expression before and after differentiation of human osteoclasts. Real-time PCR analysis of major osteoclast markers (normalized to RPLP0).

Example Assays Using Human Osteoclast Cells

Dentin slices are suitable for long-term culture as a bone model. In the user-friendly Osteo Plate assay, both von Kossa staining and TRAP (tartrate-resistant acid phosphatase) staining can be performed on the same surface.

TRAP Staining Method

The marker enzyme of osteoclasts is tartrate-resistant acid phosphatase (TRAP). TRAP activity can be readily detected using the separately available TRAP staining kit (Cat. No. PMC-AK04F, see page 7).

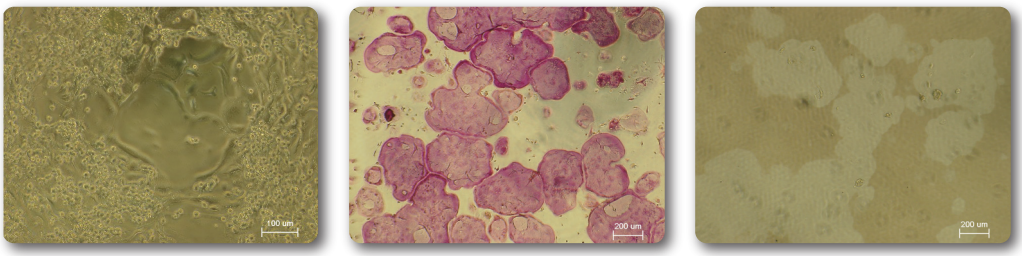


Fig. 2 Left : Osteoclasts differentiated on the surface of an Osteo Assay Plate in the presence of M-CSF and RANKL. Center : TRAP staining of osteoclasts formed on the surface of the Osteo Assay Plate Right : Von Kossa staining of resorption pits formed on the surface of the Osteo Assay Plate.

Pit Formation Assay

Osteoclasts formed on dentin slices or Osteo Assay Plates dissolve the bone matrix and generate resorption pits. The total area of these resorption pits can be quantitatively measured.

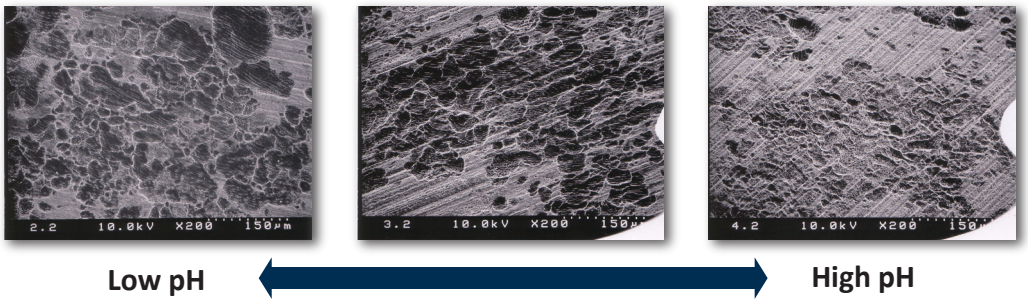


Fig. 3 pH-dependent formation of resorption pits on dentin slices.

Pit Formation Assay using Scanning Electrochemical Microscopy

Osteoclasts formed on dentin slices or Osteo Assay Plates dissolve the bone matrix and generate resorption pits. The total area of these resorption pits can be quantitatively measured.

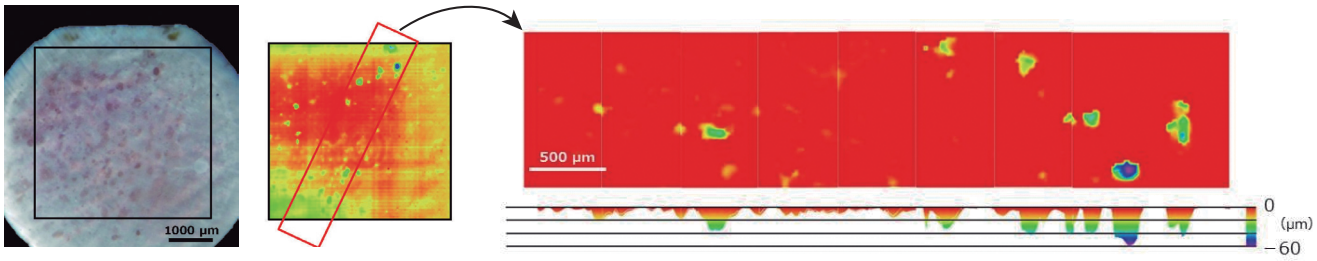


Fig. 4 General Analysis Method for Osteoclast Pit Formation Assay. The morphology of bone resorption pits formed by human osteoclasts was analyzed over a wide area. Furthermore, to examine the details of the bone resorption pit indicated by the boxed area, high-resolution measurements were performed, and the pit depth was analyzed (Fig. 5).

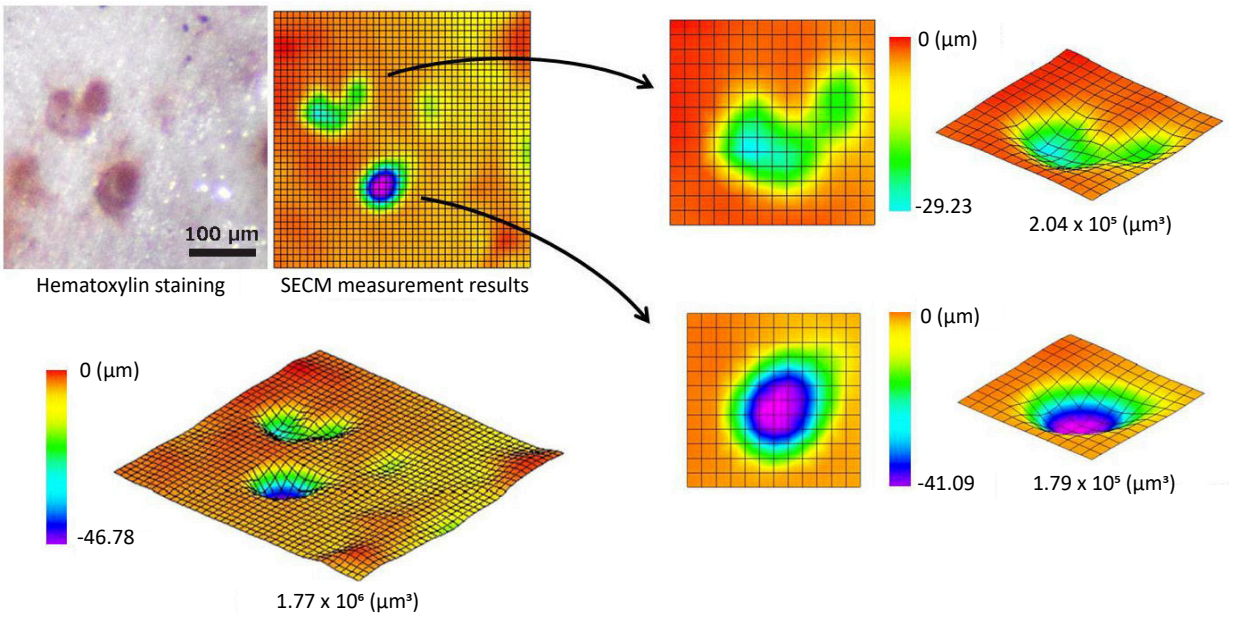


Fig. 5 Results of volume measurement of bone resorption pits. After analyzing the three-dimensional morphology of the formed bone resorption pits (Fig.4), individual resorption pit shape data were extracted, and the pit volumes were calculated.

PRODUCT NAME	CELL ORIGIN	SIZE	CATALOG NUMBER
Osteoclast Cells (human)	Human mononuclear cells	1 vial (1.5 x 10^6 cells/vial)	PMC-OSC15C
Osteoclast Cells (rat)	SD rat adult specimen	2 vials (2 x 10^6 cells/vial)	PMC-OSC12C
Osteoclast Cells (mouse)	ICR mouse adult specimen	1 vial (2 x 10^6 cells/vial)	PMC-OSC14C

PRODUCT NAME	SIZE	CATALOG NUMBER
Osteoclast Culture Medium (for human)	30 mL	PMC-OSCMHB
Osteoclast Culture Medium (for rat)	50 mL	PMC-OSCMR
Osteoclast Culture Medium (for mouse)	50 mL	PMC-OSCMM
Osteoclast Wash Medium	100 mL	PMC-OSCMW

*Osteoclast Culture Medium contains RANKL.

TRAP Staining Kit

Osteoclasts are identified by the marker Tartrate-Resistant Acid Phosphatase (TRAP). This product is suitable for staining TRAP. The staining procedure is highly simplified, making it easy to use and reliable even for first-time users.

Kit components

- Tartrate-containing Buffer 50ml
- Chromogenic Substrate 10 Vials

* Fixative (10% neutral buffered formalin) is not included.

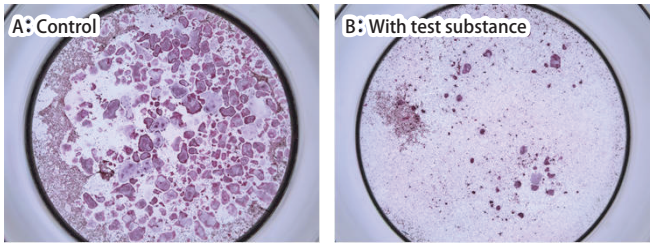


Fig 1. Bone metabolism assay (staining). Rat Osteoclasts (Cat. No. PMC-OSC12C) were exposed to the test substance for 24 hours, after which TRAP activity, a marker of osteoclasts, was evaluated using this kit based on substrate color development. In the control condition, osteoclast differentiation progressed, resulting in multinucleated giant cells with strong TRAP staining and a broad stained area. In contrast, in the presence of the test substance, osteoclast differentiation was suppressed, with weaker staining and a reduced stained area.

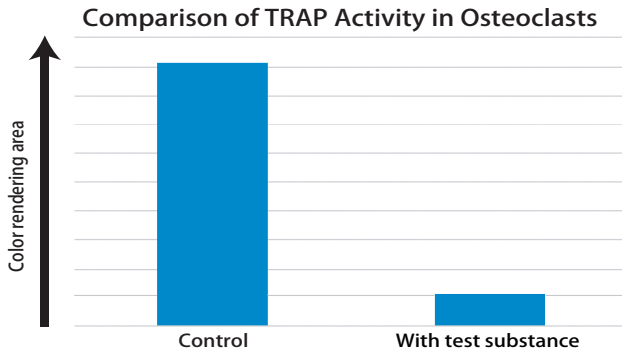


Fig 2. Figure 2. Bone metabolism assay based on quantitative analysis of TRAP-stained area. TRAP staining of rat osteoclasts was quantitatively evaluated by measuring the stained area. Results are expressed as the cumulative TRAP-positive area within the analyzed microscopic fields. The analysis revealed that the test compound significantly suppressed osteoclast differentiation.

Q1 Can this kit be used for tissue staining?

A1 This kit was developed for use with cultured cells, and no protocol for tissue staining is provided. However, there are examples of paraffin-embedded tissue staining using this kit reported in the following publications. Please refer to the methods described in those publications. Additionally, formalin-fixed samples could not be stained. We recommend using methanol-acetic acid for simultaneous fixation and decalcification. Please note that staining applications other than cultured cells are not covered by the product warranty.

1) Akio Morinobu *et., al.* (–)-Epigallocatechin-3-Gallate Suppresses Osteoclast Differentiation and Ameliorates Experimental Arthritis in Mice. *Arthritis Rheum.* 2008 Jul;58(7):2012-8. doi: 10.1002/art.23594.

2) Hong Cao *et., al.* A biodegradable porous composite scaffold of PGA/β-TCP for bone tissue engineering *Bone.* 2010 Feb;46(2):386-95. doi: 10.1016/j.bone.2009.09.031. Epub 2009 Sep 30.

3) T Ohnishi *et., al.* Involvement of Cot/Tp12 in bone loss during periodontitis *J Dent Res.* 2010 Feb;89(2):192-7. doi: 10.1177/0022034509353405.

4) Yuka Okada *et., al.* Blockade of sympathetic b-receptors inhibits Porphyromonas gingivalis-induced alveolar bone loss in an experimental rat periodontitis model *Arch Oral Biol.* 2010 Jul;55(7):502-8. doi: 10.1016/j.archoralbio.2010.04.002. DOI: 10.1016/j.archoralbio.2010.04.002

Q2 Can TRAP staining kit be used for staining osteoclasts in decalcified bone tissue?

A2 Although we have not evaluated this in our laboratory, the following reference has been reported.

Yuka Okada *et., al.* Blockade of sympathetic b-receptors inhibits Porphyromonas gingivalis-induced alveolar bone loss in an experimental rat periodontitis model *Arch Oral Biol.* 2010 Jul;55(7):502-8. doi: 10.1016/j.archoralbio.2010.04.002. DOI: 10.1016/j.archoralbio.2010.04.002

Q3 Will staining of macrophages be positive with the kit staining?

A3 Yes, macrophages will stain positive too.

PRODUCT NAME	SIZE	CATALOG NUMBER
TRAP Staining Kit	Reagents for 10 plates in 96-well format	PMC-AK04F

OSTEOGENESIS ANALYSIS TOOLS

Osteogenesis Culture Kit (mouse)

This kit contains cryopreserved cells isolated from ICR mouse bone marrow and two types of culture medium. The cells in this product can be grown using Growth Medium (Cat. No. PMC- OGCMG), and then can be differentiated into mature osteoblasts, which form calcified nodules, using Culture Medium (Cat. No. PMC- OGCMO). The media is included in this kit, but can also be purchased separately.

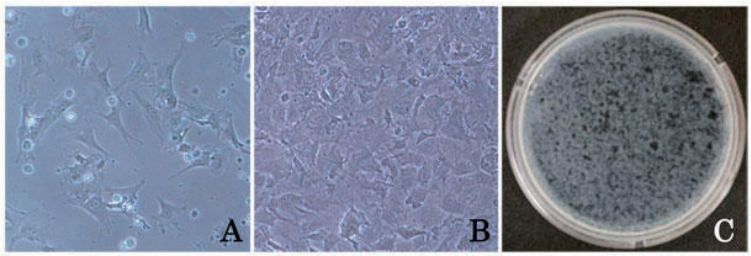


Fig. 1 Morphology of cultured cells
A:Day 0
B: confluent
C: 3 weeks after medium is changed into "Osteogenesis Culture Medium for Osteogenesis Culture kit" (35 mm dish)

Application Example

Cells are cultured according to protocol and subjected to activity staining of alkaline phosphatase or calcified nodules. The activity of alkaline phosphatase is detected by Alkaline Phosphatase Staining kit (Cat. No. PMC-AK20) and the calcium deposit is detected by Calcified nodule Staining kit (Cat. No. PMC-AK21).

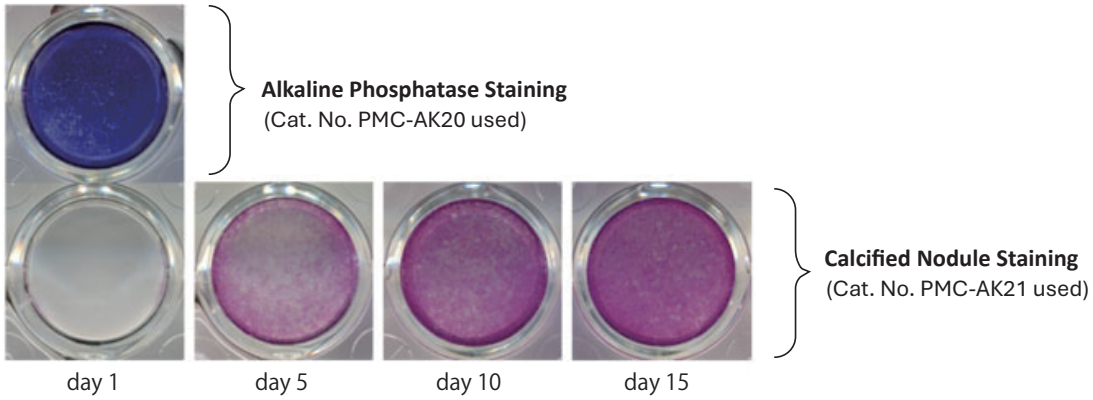


Fig. 2 The activity of alkaline phosphatase and calcium deposit of osteogenesis

PRODUCT NAME	SIZE	CATALOG NUMBER
Osteogenesis Culture Kit (mouse)	1 kit (1 x 10^6 cells/vial)	PMC-OGC11

Dedicated Media (Serum Containing Media)

PRODUCT NAME	SIZE	CATALOG NUMBER
Growth Medium for Osteogenesis Culture Kit	250 mL	PMC-OGCMG
Osteogenesis Culture Medium	250 mL	PMC-OGCMO

Osteoblast Cells (Rat, Mouse)

Although many osteoblast-like immortalized cell lines have been established, they do not necessarily fully reproduce all osteoblast functions observed in vivo under *in vitro* conditions. These Osteoblast Cells are primary cultured cells that migrate out from rat/mouse (aged 1-4 days) calvarial bone fragments and retain physiological functions characteristic of osteoblasts *in vivo*. Based on the evaluation of alkaline phosphatase activity staining, these cells have been confirmed to be passaged at least twice.

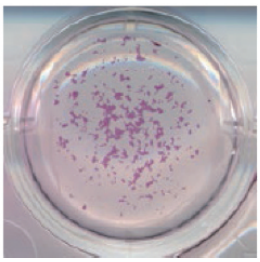


Fig. 1 Stained image of calcified osteoblasts. Confluent rat osteoblasts were cultured in Osteogenic Differentiation Medium (Cat. No. PMC-OGCMO) for 28 days and subsequently stained using a Mineralization Staining Kit (Cat. No. PMC-AK21). Cells were cultured in a 12-well plate.

Features

- Retain physiological functions characteristic of osteoblasts in vivo

PRODUCT NAME	SIZE	CATALOG NUMBER
Osteoblast from Cranial Bone (SD rat)	1 vial (1 x 10^6 cells/vial)	PMC-OBC02C
Osteoblast from Cranial Bone (ICR mouse)	1 vial (1 x 10^6 cells/vial)	PMC-OBC12C
Osteoblast Culture Medium	500 mL	PMC-OBCM

Alkaline Phosphatase Staining Kit

The Alkaline Phosphatase Staining Kit is used for determining alkaline phosphatase activity with ease. Osteoclasts express TRAP as a characteristic enzymatic marker, whereas osteoblasts are characterized by alkaline phosphatase activity. Osteoblasts, as well as cells differentiated toward the osteogenic lineage from bone marrow–derived cells, can be detected by alkaline phosphatase staining.

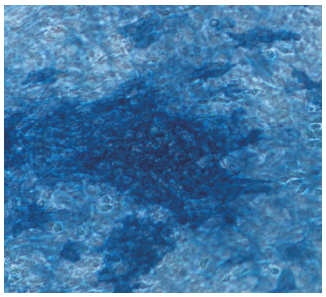


Fig. 1 Rat osteoblasts stained with Alkaline Phosphatase Staining Kit. Rat Osteoblasts (Cat.No. PMC-OBC02) were cultured in Osteogenesis Culture Medium and the activity of alkaline phosphatase was detected by this kit.

PRODUCT NAME	SIZE	CATALOG NUMBER
Alkaline Phosphatase Staining Kit	1 kit (with 10 vials*)	PMC-AK20

*One kit contains reagents for 10×12-well plates.

Calcified Nodule Staining Kit

The mineralization staining kit contains Alizarin Red S, a dye that binds specifically to calcium, and enables simple and reliable staining of calcified bone nodules. This kit can stain 10, 24-well plates.

PRODUCT NAME	SIZE	CATALOG NUMBER
Calcified Nodule Staining Kit	1 set (with 10 vials*)	PMC-AK21

*One kit contains reagents for 10×24-well plates.

BONE MARROW RESEARCH TOOLS

Bone Marrow-derived, Compact Bone-derived Mesenchymal Stem Cells

Bone marrow–derived mesenchymal stem cells are obtained from adult SD rat bone marrow. The isolated cell population is processed by density gradient centrifugation, and the collected fraction is subsequently expanded through one passage.

Compact bone–derived mesenchymal stem cells are derived from adult SD rat cortical bone following collagenase digestion, and the isolated cells are expanded through two passages. Alternatively, they may be derived from adult C57BL/6 mouse cortical bone after collagenase digestion and expanded through three passages.

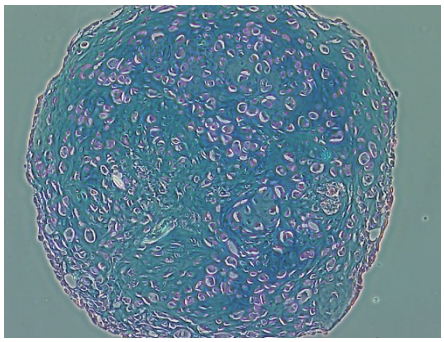


Fig 1. Cartilage-differentiated Rat Bone Marrow-derived Mesenchymal Stem Cells (Cat. No. PMC-MSB01C) stained with Alcian blue.

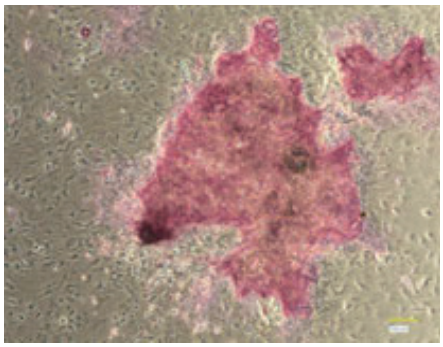


Fig 2. Calcified staining of one-differentiated Mouse Compact Bone-derived Mesenchymal Stem Cells (Cat. No. PMC-BMMW) using a Calcification Staining Kit (Cat. No. PMC-AK21).

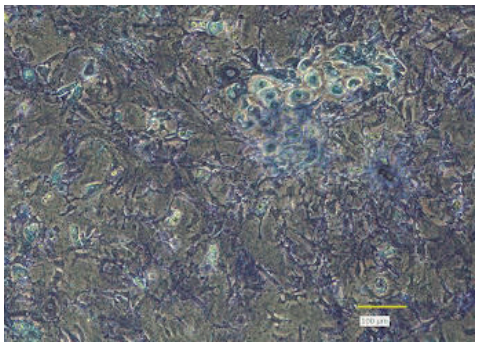


Fig 3. Rat Osteoblasts (Cat. No. PMC-OB-C02C) were cultured in Osteogenesis Culture for Osteogenesis Culture kit and the activity alkaline phosphatase was detected by Alkaline Phosphatase Staining Kit (Cat. No. PMC-AK20).

PRODUCT NAME	SIZE	CATALOG NUMBER
Bone Marrow-derived Mesenchymal Stem cells (BMMSCs) (SD Rat)	1 vial (0.5 x 10^6 cells)	PMC-MSB01C

PRODUCT NAME	SIZE	CATALOG NUMBER
Compact Bone-derived Mesenchymal Stem Cells (CBMSCs) (SD Rat)	1 vial (1 x 10^6 cells)	PMC-MSCB01C
Compact Bone-derived Mesenchymal Stem Cells (CBMSCs) (C57BL/6 Mouse)	1 vial (1 x 10^6 cells)	PMC-MSCB11C

Dedicated Growth and Differentiation Medium

PRODUCT NAME	SIZE	CATALOG NUMBER
BMMSC Growth Medium	200 mL	PMC-MSB-GM
CBMSC Growth Medium	250 mL	PMC-MSCB-GM
CBMSC Adipogenic Differentiation Medium	100 mL	PMC-MSCB-ADDM
CBMSC Adipogenic Maintenance Medium	250 mL	PMC-MSCB-ADMM
Osteogenesis Culture Medium	250 mL	PMC-OGCMO
BMMSC / CBMSC Chondrogenic Differentiation Medium	50 mL	PMC-MSC-CHB

Monocyte Precursor Cells (rat)

This product contains frozen precursor cells of monocytes from bone marrow. They can differentiate into monocytes using a specialized medium containing M-CSF (Macrophage Colony Stimulating Factor).

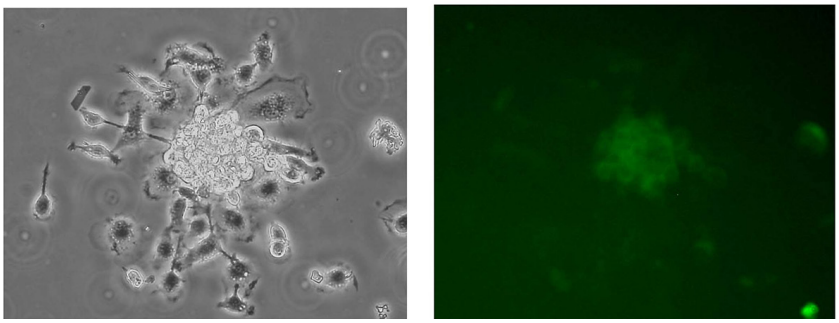


Fig.1 Cell morphology. Left: Phase contrast. Right : Anti-Mac1 FITC Staining.

PRODUCT NAME	SIZE	CATALOG NUMBER
Bone Marrow Monocyte Precursor Cell (rat)	1 vial (2 x 10^6cells/vial)	PMC-BMMC

Dedicated Culture Medium and Wash Medium

PRODUCT NAME	SIZE	CATALOG NUMBER
Monocyte Culture Medium	25 mL	PMC-BMMG
Monocyte Wash Medium	50 mL	PMC-BMMW

CARTILAGE RESEARCH TOOLS

Rabbit Chondrocyte

These primary cultured chondrocytes (frozen cells) are derived from JW rabbit articular cartilage tissue, without loss of function. This product can be passaged only once.

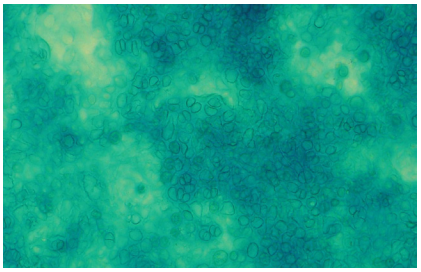


Fig 1. Alcian Blue staining of rabbit chondrocytes.

PRODUCT NAME	SIZE	CATALOG NUMBER
Chondrocytes (rabbit)	1 vial (2 x 10^6cells/vial)	PMC-CHC04C

PRODUCT NAME	SIZE	CATALOG NUMBER
Chondrocyte Growth Medium	500 mL	PMC-CHCG
Chondrocyte Differentiation Medium	500 mL	PMC-CHCM
Collagen Coating Solution	100 mL	PMC-SCO

COSMO BIO USA
Inspiration for Life Science

TEL: +1 (760) 431-4600
(Available Monday – Friday, 9:00 AM – 5:00 PM (PT)

Address: Cosmo Bio USA
2792 Loker Avenue West, Suite 101
Carlsbad, CA 92010 USA

Website: cosmobiousa.com