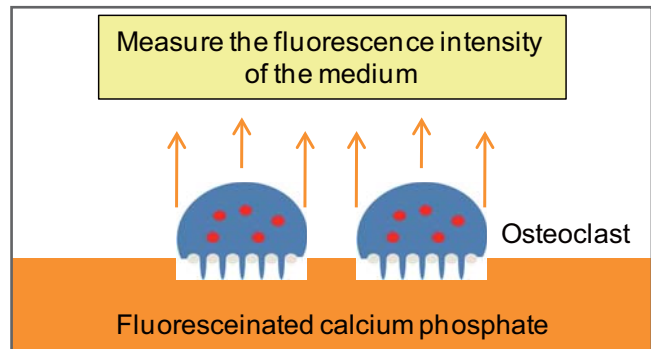


BONE RESORPTION ASSAY

KIT & PLATE

Easy-to-use and Fast Results!

Measuring bone resorption activity with a fluoresceinated calcium phosphate-coated plate provides a rapid evaluation system compared to conventional pit formation assays. This kit can be used for studies of bone metabolism (e.g. osteoporosis) and drug assessment.



Product Features

- Bone resorption activity is evaluated by the fluorescence intensity of the medium.
- Cell morphology can be microscopically observed.
- Pit area can be analyzed after the assay.
- Sterilized components are ready-to-use for the assay.
- **BONE RESORPTION PLATE** is an alternative to ivory slice.

Discription	Cat. No.	Quantity
BONE RESORPTION ASSAY KIT 24	CSR-BRA-24KIT	1 kit
BONE RESORPTION ASSAY KIT 48	CSR-BRA-48KIT	1 kit
BONE RESORPTION ASSAY KIT 48x2	CSR-BRA-48X2KIT	2x1 kit
BONE RESORPTION ASSAY PLATE 24	CSR-BRA-24P	1 plate
BONE RESORPTION ASSAY PLATE 48	CSR-BRA-48P	1 plate
BONE RESORPTION ASSAY PLATE 48x2	CSR-BRA-48X2P	2x1 plate
BONE RESORPTION ASSAY FACS	CSR-BRA-FACS1	13 mL
BONE RESORPTION ASSAY BUFFER	CSR-BRA-B1	10 mL



BONE RESORPTION ASSAY KIT & PLATE

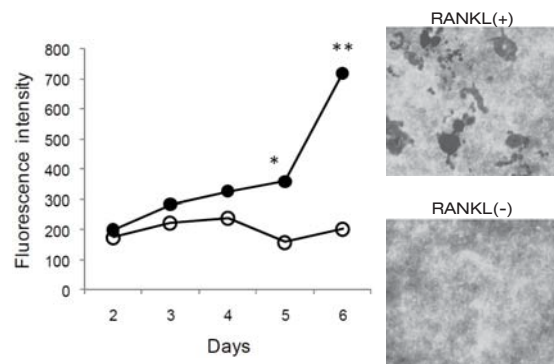
Example Drug assessment with macrophage cell line RAW264

1. Add BONE RESORPTION ASSAY FACS to each well of the BONE RESORPTION ASSAY PLATE and incubate at 37°C for 1-2 hours.
2. After washing the plate with PBS(-) and medium, inoculate the macrophage cell line RAW264 into each well. Culture cells in the presence of an osteoclastic differentiation inducer, such as RANKL, and drugs to be evaluated (for about 5-6 days).
3. Transfer conditioned medium to a 96-well microplate and add BONE RESORPTION ASSAY BUFFER to each well. Measure the fluorescence intensity at an excitation wavelength of 485 nm and emission wavelength of 535 nm.
4. To measure the pit area, remove cells by treating wells with 5% sodium hypochlorite. After washing the plates, photograph the pit using a microscope and measure the area with an image analyzing software.

Assay Precautions

- 1) Use phenol red-free culture medium.
- 2) Protect from strong light as much as possible.

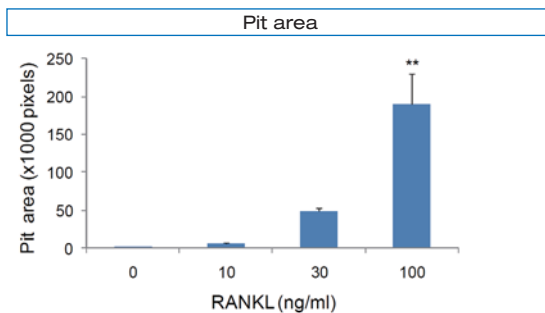
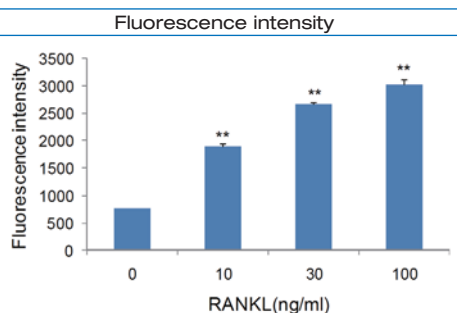
Osteoclast differentiation induced by RANKL



Osteoclast differentiation of RAW264 cells was induced by RANKL (100 ng/mL) and evaluated by measuring the fluorescence intensity of supernatant of the medium. With RANKL, pit formation and increased fluorescence intensity were observed while "without RANKL" showed steady fluorescence intensity. (●: with RANKL, ○: without RANKL, *: $p < 0.05$, **: $p < 0.001$) Photographs show CaP-coated plates (on day 6)

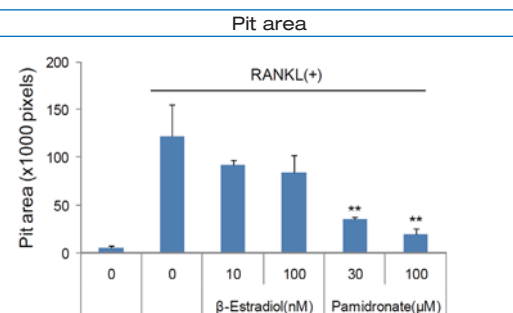
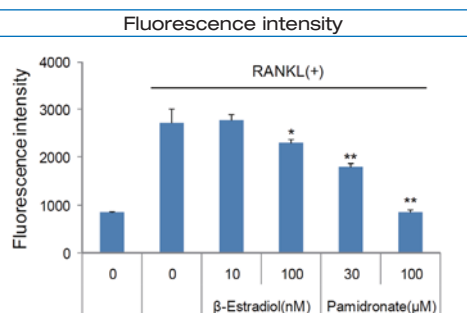
Reference Data Results using the macrophage cell line RAW264 (Miyazaki T. *et al*, *Anal Biochem*. 410(1): 7-12, 2011)

Bone resorption activity and RANKL concentration



RANKL-dependent increases of fluorescence intensity (left) and pit area (right) (mean \pm S. D., $n = 3$, **: $p < 0.001$).

Evaluation of test substances for treating osteoporosis



The inhibitory effects of Pamidronate and β -Estradiol on the resorption of CaP induced by RANKL (100 ng/mL) were evaluated by fluorescence intensity (left) and pit area (right) (mean \pm S. D., $n = 3$, *: $p < 0.05$, **: $p < 0.001$).

