



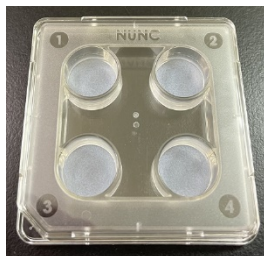
# BONE RESORPTION ASSAY PLATE

(4well 24well 48well 96well)

This product is a calcium phosphate (CaP)-coated plate used to measure the bone resorption activity of osteoclasts. The plate is coated with a synthetic CaP (carbonate apatite), similar to that of natural apatite, which is able to be used as an alternative to dentin discs.

## 1) Specifications

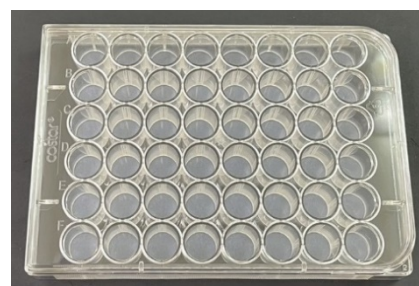
- (A) A 4-well plate coated with a synthetic CaP (gamma sterilized, Cat.BRA-4P)
- (B) A 24-well plate coated with a synthetic CaP (gamma sterilized, Cat.BRA-24P)
- (C) A 48-well plate coated with a synthetic CaP (gamma sterilized, Cat.BRA-48P)
- (D) A 96-well plate coated with a synthetic CaP (gamma sterilized, Cat.BRA-96P)
- (E) A 96-well plate (8-well strip) coated with a synthetic CaP (gamma sterilized, Cat.BRA-S96P)



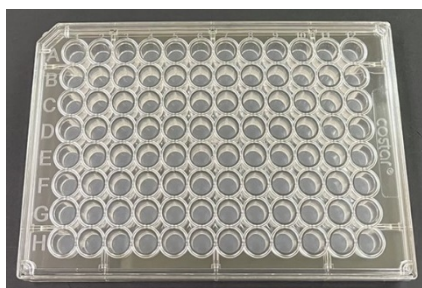
A : BRA-4P



B : BRA-24P



C : BRA-48P



D : BRA-96P



E : BRA-S96P

## 2) Example of use

- A: Wash each well of the 4-well plate with 1 mL of culture medium.
  - B: Wash each well of the 24-well plate with 1 mL of culture medium.
  - C: Wash each well of the 48-well plate with 0.5mL of culture medium.
  - D: Wash each well of the 96-well plate with 0.2mL of culture medium.
  - E: Wash each well of the 96-well plate (8-well strip) with 0.2mL of culture medium.
- (1) Inoculate RAW264 or RAW264.7 cells into each well in culture medium (DMEM/F-12 or MEM $\alpha$  containing 10% FBS). Add an inducer of osteoclastic differentiation, such as RANKL (100 ng/mL) and the test substances to be evaluated.
- A: 4-well plate 1x10<sup>4</sup> cells/mL; 1 mL/well
  - B: 24-well plate 1x10<sup>4</sup> cells/mL; 1 mL/well
  - C: 48-well plate 5x10<sup>3</sup> cells/mL; 0.5mL/well
  - D: 96-well plate 2x10<sup>3</sup> cells/mL; 0.2mL/well
  - D: 96-well plate (8-well strip) 2x10<sup>3</sup> cells/mL; 0.2mL/well
- (2) On day 3, change the medium with freshly made medium (containing RANKL and drugs). This step may be eliminated. However, the induction of osteoclastic differentiation by RANKL would be reduced.
- (3) On day 5 or 6, remove the conditioned medium from each well and treat the wells with 5% sodium hypochlorite for 3-5 minutes (Do not leave the plate for more than 5 minutes in the 5% sodium hypochlorite). After washing the

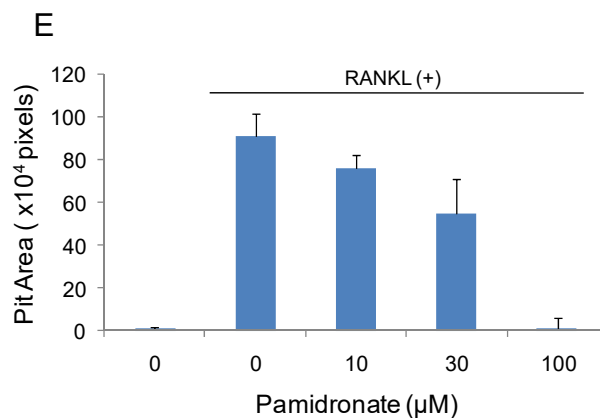
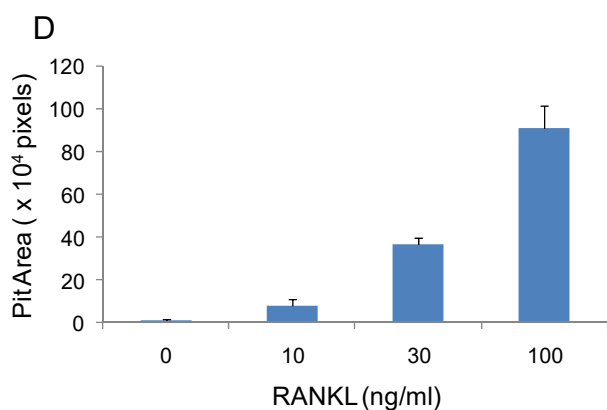
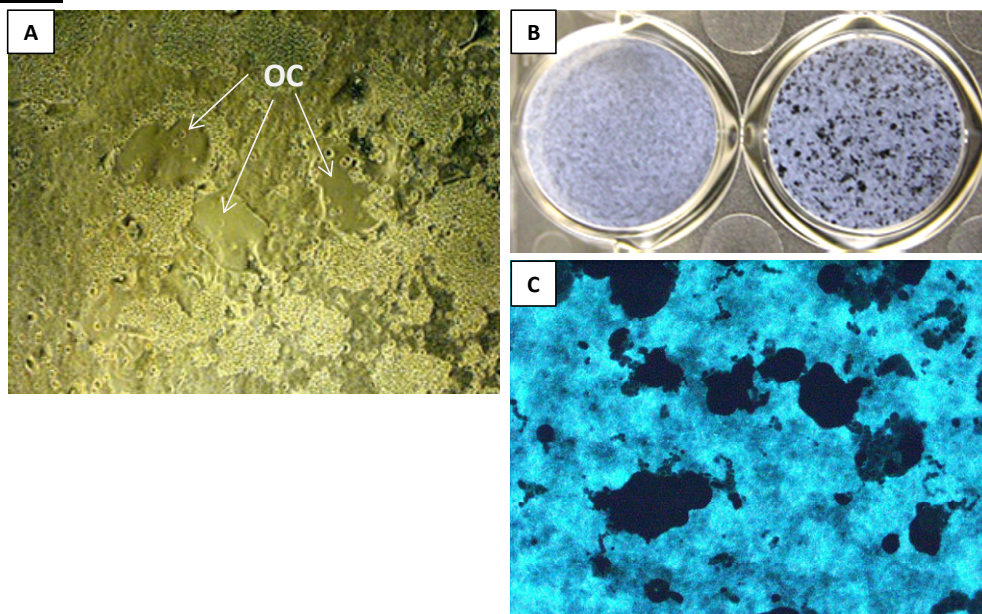


plate with water and then drying it, photograph the regions in each well using a microscope and measure the pit area with image analyzing software.

### 3) Assay precautions

- (1) To stimulate cells, we recommend a RANKL concentration  $\geq 100$  ng/mL.
- (2) This product is for research use only, and not for use in diagnostic or therapeutic procedures.

### 4) Expected Results



- A. A phase-contrast micrograph of RAW264 cells (day 6) cultured in CaP-coated plates stimulated with RANKL (Oriental Yeast Co., Ltd., Tokyo, Japan; 100 ng/mL). Osteoclast-like cells (OC) were observed.
- B. Photograph of the plate after removing cells. Pits can be observed macroscopically (Left: without RANKL; Right: with RANKL).
- C. Micrograph of the pits in a CaP-coated plate (with RANKL).
- D. RANKL-dependent increase of the pit area (mean  $\pm$  S.D., n = 3).
- E. The inhibitory effect of the bisphosphonate, Pamidronate, on CaP resorption induced by RANKL (100 ng/mL).

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Manufactured by **PG**  
Research



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