COSMO BIO CO., LTD.
Inspiration for Life Science

BONE RESORPTION ASSAY PLATE

(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 24-well plate coated with a synthetic CaP (gamma sterilized, Cat.BRA-24P)

(B) A 48-well plate coated with a synthetic CaP (gamma sterilized, Cat.BRA-48P)

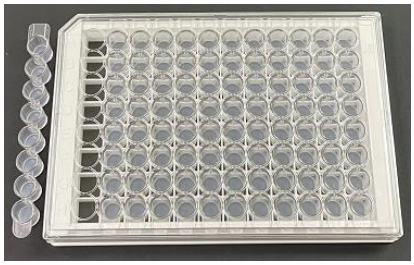
(C) A 96-well plate coated with a synthetic CaP (gamma sterilized, Cat.BRA-96P))

(D) A 96-well plate (8-well strip) coated with a synthetic CaP (gamma sterilized, Cat.BRA-S96P)

A: BRA-24P

B: BRA-48P

C: BRA-96P



D: BRA-S96P

2) Example of use

(1) **A:** Wash each well of the 24-well plate with 1 mL of culture medium.

B: Wash each well of the 48-well plate with 0.5mL of culture medium.

C: Wash each well of the 96-well plate with 0.2mL of culture medium.

D: Wash each well of the 96-well plate (8-well strip) with 0.2mL of culture medium.

(2) Inoculate RAW264 or RAW264.7 cells into each well in culture medium (DMEM/F-12 or MEMα containing 10% FBS). Add an inducer of osteoclastic differentiation, such as RANKL (100 ng/mL) and the test substances to be evaluated.

A: 24-well plate $1x10^4$ cells/mL; 1 mL/well
B: 48-well plate $5x10^3$ cells/mL; 0.5mL/well
C: 96-well plate $2x10^3$ cells/mL; 0.2mL/well
D: 96-well plate (8-well strip) $2x10^3$ cells/mL; 0.2mL/well

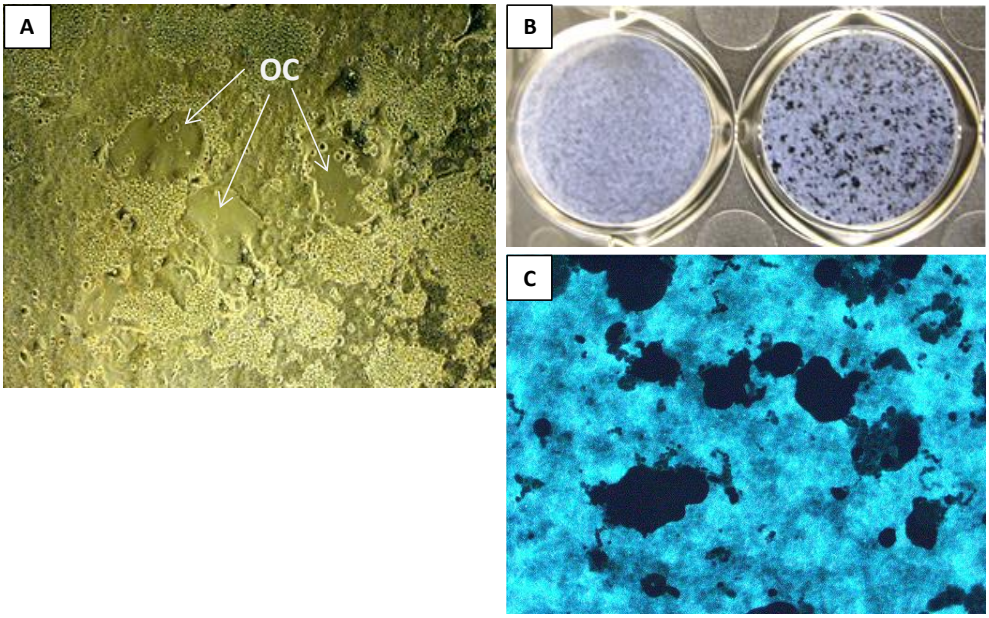
(3) On day 3, change the medium with freshly made medium (containing RANKL and drugs). This step may be eliminated. However, the induction of osteoclastic differentiation by RANKL would be reduced.

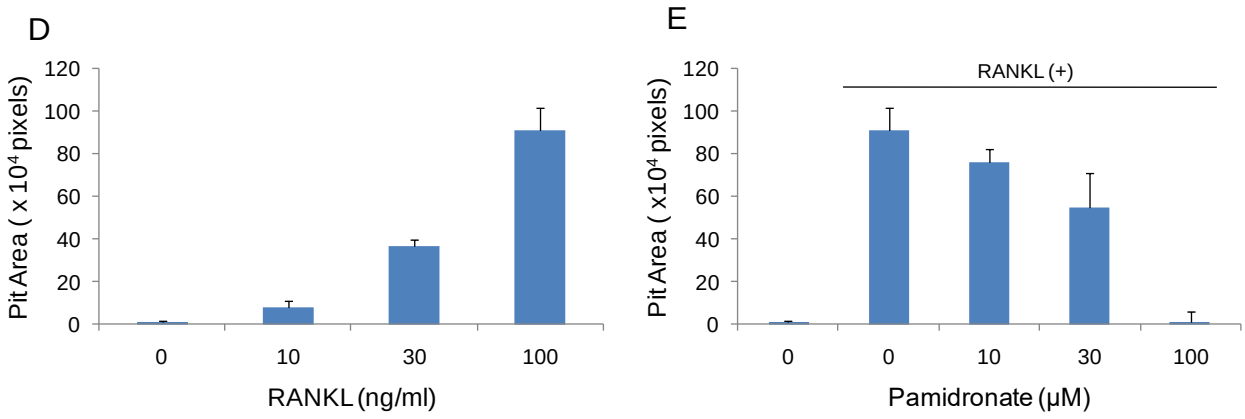
(4) On day 5 or 6, remove the conditioned medium from each well and treat the wells with 5% sodium hypochlorite for 3-5 minutes (Do not leave the plate for more than 5 minutes in the 5% sodium hypochlorite). After washing the plate with water and then drying it, photograph the regions in each well using a microscope and measure the pit area with image analyzing software.

3) Assay precautions

(1) To stimulate cells, we recommend a RANKL concentration ≥ 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 ± 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

COSMO BIO CO., LTD.
【JAPAN】
TOYO EKIMAE BLDG. 2-20, TOYO 2-CHOME,
KOTO-KU. TOKYO 135-0016, JAPAN
Phone: +81-3-5632-9610
FAX: +81-3-5632-9619
URL: https://www.cosmobio.co.jp/

COSMO BIO USA
【Outside Japan】
2792 Loker Ave West, Suite 101
Carlsbad, CA 92010, USA
email: info@cosmobiousa.com
Phone/FAX: (+1) 760-431-4600
URL: www.cosmobiousa.com