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Research

TRAP Assay Set

Tartrate-Resistant Acid Phosphatase (TRAP) is widely recognized as a marker of osteoclast differentiation and is released into the medium during differentiation in osteoclast cultures[1)2)]. This product is a set of reagents to detect TRAP activity in cultured osteoclasts and can be used to evaluate osteoclast differentiation and drugs for osteoporosis. A TRAP substrate solution containing p-nitrophenyl phosphate is added to the culture supernatant or fixed cells, and the p-nitrophenol produced by the reaction of the TRAP enzyme is detected by absorbance at 405 nm in this simple method.

1） Composition of reagent sets

①TRAP Assay Substrate Solution（Cat.TRAP-S1, 15mL, 2 bottles）
Citrate buffer (pH 5.5) containing p-nitrophenyl phosphate and sodium tartrate

②TRAP Assay Stop Solution（Cat.TRAP-E1, 15mL, 2 bottles）
Sodium hydroxide solution (0.1 mol/L)

2） Example of us

TRAP activity of culture supernatants

(1) Inoculate RAW264.7 cells into each well in culture medium (MEMα containing 10% FBS). Add an inducer of osteoclastic differentiation, such as RANKL (100 ng/mL) and the test substances to be evaluated.
96well : 2,000cells/0.2mL/well
48well : 5,000cells/0.5mL/well
24well : 10,000cells/1mL/well
(2) After 72 or 96 hours of incubation, collect 50 µL of culture supernatant in a 96-well plate for absorbance measurement.
(3) Add 50 µL of TRAP Assay Substrate Solution to the supernatant of each well, mix and react at 37°C for 30 min to 1 h (light-shielded).
(4) Add 50 µL of TRAP Assay Stop Solution to each well and stir (colour develops at this point).
(5) Measure absorbance at 405 nm.

TRAP activity of cells

(1) After cultivation, remove the medium from each well of the plate, add 10% neutral buffered formalin solution and fix the cells for 10 min at room temperature.
(2) Remove the formalin solution and wash the cells in each well twice with purified water (0.2-1 mL).
(3) Add the following volumes of TRAP Assay Substrate Solution and react at 37°C for 30 min to 1 h (light-shielded).
96-well plate: 100 µL/well
48-well plate: 250 µL/well
24-well plate: 500 µL/well
(4) Add the same volume of TRAP Assay Stop Solution as Substrate Solution (colour develops at this point) and stir gently.
(5) After collecting 100 µL of the reaction solution into a 96-well plate, measure the absorbance at 405 nm.

3） Assay precautions

(1) When using this reagent, always wear protective equipment such as glasses and gloves and take care to avoid contact with the human body.
(2) After thawing this reagent, stir it well before use.
(3) When storing residual reagents after measurement use, they should be frozen (-20°C). It has been confirmed that there are no quality problems after refreezing and thawing three times.
(4) When reacting with TRAP substrates, shield from light and adjust the reaction time to suit your culture system. Colour develops when Stop Solution is added.
(5) Reactions can be carried out at room temperature, but the overall absorbance will be lower than at 37 °C.
(6) Can also be used for culture supernatants containing phenol red.
(7) When using calcium phosphate coated plates (Bone Resorption Assay Plate) to measure TRAP activity of cells, use the supernatant after centrifugation at 2,000 x g for 3 min, as the addition of Stop Solution causes cloudiness.

4） References

1. Alatalo SL, Clin Chem, 2000, 46(11):1751-1754.
2. Lv Y, Exp Ther Med, 2015, 9(1):143-146.

5） Expected Results

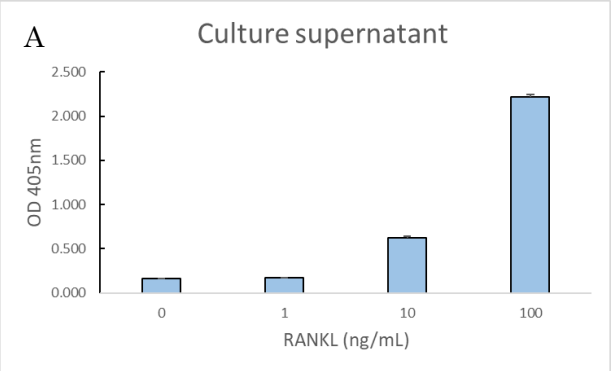

Fig.1 RAW264.7 cells (48-well plate, 10% FBS/MEMα) were incubated with RANKL for 96 h upon seeding, and the culture supernatant and cells were collected. A: 50 µL of Substrate Solution to 50 µL of culture supernatant, B: 250 µL of Substrate Solution was added to the fixes cells, and allowed to react for 1 h at 37 °C. After adding Stop Solution, absorbance at 405 nm was measured. (Mean±SD, n=4)

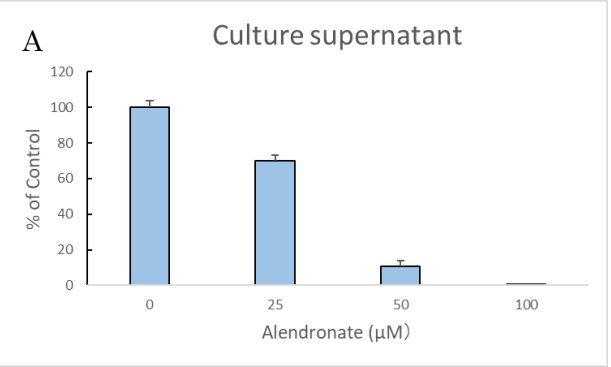

Fig.2 RAW264.7 cells (96-well plate, 10% FBS/MEMα) were incubated with RANKL(100ng/mL) and various concentrations of Alendronate for 72 h upon seeding, and the culture supernatant and cells were collected. A: 50 µL of Substrate Solution to 50 µL of culture supernatant, B: 100 µL of Substrate Solution was added to the fixed cells, and allowed to react for 1 h at 37 °C. After adding Stop Solution, absorbance at 405 nm was measured. (Mean±SD, n=4)

Catalog Number	Product Name	Specification	Price	Storage
TRAP-SET	TRAP Assay Set	1 set (TRAP-S1, TRAP-E1: 2 each)	¥21,000	−20℃

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