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NBP2-31223 Protocol

Assay Instruction Manual (NBP2-31223)

Osteoclast Formation Assay:

Treatment of mouse spleen cells with soluble RANKL together with MCSF induces osteoclast formation from spleen cells. This procedure is now being routinely used by various laboratories to study induction of osteoclast differentiation.
1. Culture mouse spleen or bone marrow cells (7 x 10^5 cells) obtained from 6- to 15-week old male mice or RAW264.7 (1 x 10^5/well) (a mouse macrophage cell line) for 4-5 days in a 24-well plate in DMEM medium containing 10% fetal calf serum (FCS), 20 ng/ml human M-CSF (R&D Biosystems, Inc.), 30 ng/ml of RANKL.
2. Add different amount of control and T6DP peptides at the beginning of culture.
3. On day 3, change the medium with additives listed above including T6DP.
4. Evaluate differentiation of spleen cells, bone marrow cells, and RAW cells into osteoclasts by measuring tartrate-resistant acid phosphate (TRAP) activity. TRAP positive cells with more than 3 nuclei are considered as osteoclasts.
TRAP-positive cells are detected between day 7 and 10 (BM) or on day 5 (RAW264.7).