

Bone Resorption Assay

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[Abstract] The Bone resorption assay provides an easy to use protocol for quantitatively measuring *in vitro* osteoclast-mediated bone resorption. Osteoclasts can be seeded onto the bone slices and formation of resorption pits can be quantified via toluidine blue staining (Scholtysek *et al.*, 2013).

Materials and Reagents

1. Osteoclasts
2. Bone slices (IDS PLC, catalog number: DT-1BON1000-96)
3. Isopropanol
4. Toluidine blue O (Sigma-Aldrich, catalog number: 198161)
5. MilliQ water
6. 96 well plates for cell culture (Greiner Bio-One GmbH, catalog number: 650185)

Equipment

1. OsteoMeasure System (http://www.osteometrics.com/product_info.htm)
2. Ultrasonic water bath

Procedure

1. Osteoclasts were plated (250,000 cells/well) in the presence and absence of calvarial osteoblasts in 96-well plates, previously equipped with bone slices.
2. Cells were cultured in appropriate media for at least 14 days at 37 °C.
3. After this time cells were washed twice with PBS and removed from bone slices via ultrasonication in 250 µl of 70% isopropanol for at least 15 min at high power.

Note: A volume of 250 µl of 70% isopropanol before ultrasonication makes it easier to get rid of the cells.

4. Resorption pit formation was visualized by 100 μ l toluidine blue (1%; dissolved in water) staining for 2 min at RT.
5. The slices were then rinsed with 250 μ l MilliQ water at least 5 times to wash out residues.
6. Resorption pits are now stained in dark blue (Figure 1) and resorption area can be quantified via Osteomeasure System or resorption pits/well can be counted via light microscopy.

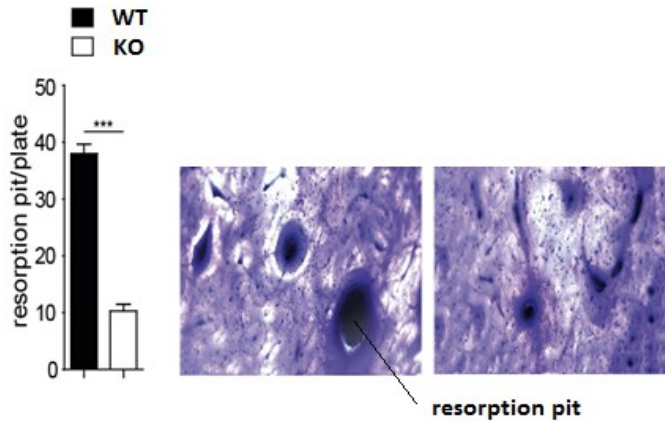


Figure 1. Resorption pit formation

Acknowledgments

This protocol was adapted from the previously published paper Scholtysek *et al.* (2013).

References

1. Scholtysek, C., Katzenbeisser, J., Fu, H., Uderhardt, S., Ipseiz, N., Stoll, C., Zaiss, M. M., Stock, M., Donhauser, L., Bohm, C., Kleyer, A., Hess, A., Engelke, K., David, J. P., Djouad, F., Tuckermann, J. P., Desvergne, B., Schett, G. and Kronke, G. (2013). [PPARbeta/delta governs Wnt signaling and bone turnover](#). *Nat Med* 19(5): 608-613.