

## 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

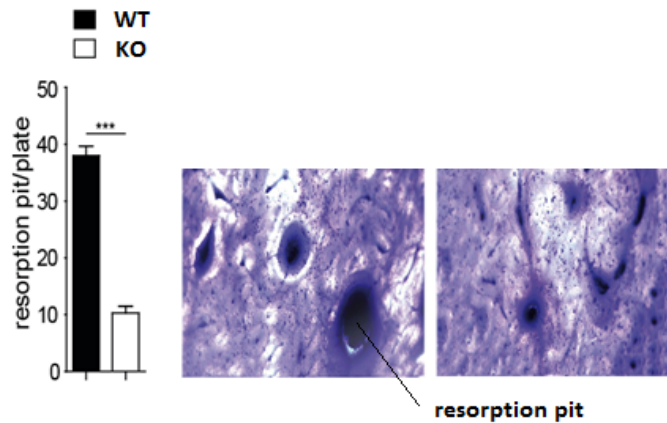
1. OsteoMeasure System ([http://www.osteometrics.com/product\\_info.htm](http://www.osteometrics.com/product_info.htm))
2. Ultrasonic water bath

### 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  $\mu$ l toluidine blue (1%; dissolved in water) staining for 2 min at RT.
5. The slices were then rinsed with 250  $\mu$ 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.