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Tartrate Resistant Acid Phosphatase (TRAP) Staining of Osteoclasts on BD BioCoat™ Osteologic™ Discs and MultiTest Slides

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Introduction

The BD BioCoat™ Osteologic™ Bone Cell Culture System consists of sub-micron synthetic calcium phosphate thin films coated onto various culture vessels. This system has been used as an alternative method for compound screening for direct assessment of osteoclast¹⁻⁴ and osteoblast activity *in vitro*. The thin film design permits easy and reliable quantification of results.

The use of BD BioCoat Osteologic Discs and MultiTest Slides to characterize and measure osteoclast mediated bone resorption provide an easy method to study osteoclastogenesis and function for both drug development and basic research purposes. A commonly used marker of osteoclast identity is the enzymatic activity of tartrate resistant acid phosphatase (TRAP). Described here is a TRAP staining method that has been optimized for use with the BD BioCoat Osteologic Discs and MultiTest Slides.

Methods

Primary considerations when performing this assay are the time and temperature of the reagents. The thin film coating is stable at physiological pH values, but will slowly dissolve at pH values lower than 6.5. The speed of dissolution increases as the pH is lowered and the incubation time is extended. This is critical in performing this assay since the buffer required for optimal acid phosphatase activity is pH 5.0. The recommended combination of reagents promotes rapid development of the reaction and high intensity color.

Materials

- **10% Glutaraldehyde Solution**
 - Prepare by adding 40 ml glutaraldehyde stock solution [25% aqueous solution (Sigma Cat. No. G-5882)] to 60 ml H₂O
- **PBS Solution** [Gibco Cat. No. 10010-023]
- **Fast Red Violet LB Salt** [Sigma Cat. No. F-3381]
- **0.2 M Sodium Acetate Solution**
 - Prepare by adding 16.4 g Sodium Acetate [Fisher Cat. No. S210B, FW: 82.03] per liter of H₂O
- **0.2 M Acetic Acid Solution**
 - Prepare by adding 11.5 ml of Glacial Acetic Acid [BDH Labs Cat. No. ACS003-78] to 988.5 ml H₂O
- **0.3 M Sodium Tartrate**
 - Prepare by adding 6.9 g Sodium Tartrate [Sigma Cat. No. S-8640, FW: 230.1] per 100 ml of H₂O
- **Triton® X-100** [Sigma Cat. No. T-8787]
- **Naphtol AS-MX Phosphate Disodium Salt**
 - Prepare fresh each time
 - Prepare by adding 10 mg Phosphate Disodium Salt [Sigma Cat. No. N-5000, FW: 415.3] per ml of H₂O
- **Hoechst Stain #33342 (Bisbenzimidide)** [Sigma Cat. No. B-2261, FW: 561.9]
- **0.1 M Acetate Buffer**
 - 35.2 ml 0.2 M Sodium Acetate Solution
 - 14.8 ml 0.2 M Acetic Acid Solution
 - 50 ml water
- **TRAP Buffer pH 5.0**
 - Prepare fresh each time
 - 50 ml 0.1 M Acetate Buffer
 - 10 ml 0.3 M Sodium Tartrate
 - 1 ml 10 mg/ml Naphtol AS-MX phosphate
 - 100 µl Triton X-100
 - 38.9 ml Milli-Q water
- **TRAP Stain**
 - Prepare fresh each time
 - Dissolve 0.3 mg Fast Red Violet LB stain per ml of TRAP buffer prewarmed in 37°C water bath

continued

Materials (continued)

Fluorescent Nuclei Stain (Optional)

- **Stock Solution**
 - Dissolve 0.5 mg Hoechst stain in 1 ml PBS
 - Store at -20°C
- **Working Solution**
 - Add .1 ml of the stock solution to 9.9 ml of PBS for a final concentration of 5 µg/ml solution

Procedures

- Remove medium and wash cells with PBS
- Ensure TRAP stain is prewarmed to 37°C
- Fix cells with 10% Glutaraldehyde for 15 minutes at 37°C
- Wash cells 2x with PBS prewarmed to 37°C
- Treat cells with TRAP stain for 5-10 minutes at 37°C (cover surface of disc with stain with approximately 300 µl)
- Remove TRAP stain and wash with PBS
- Observe under standard light microscopy

Fluorescent Nuclear Stain (Optional)

- Stain with Hoechst stain for 10 minutes at room temperature
- Wash with water and store in the dark at 4°C
- Use a microscope equipped with a UV light source to view cells

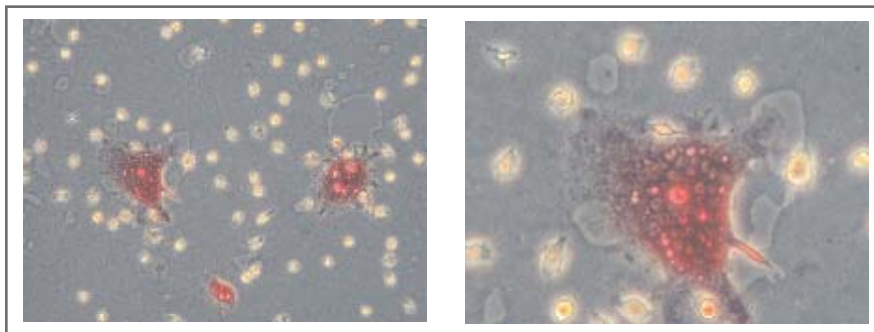


Figure 1: Rat bone marrow cells cultured for eight days on BD BioCoat™ Osteologic™ Discs.

Results

BD BioCoat™ Osteologic™ Discs and MultiTest Slides were viewed under standard light microscopy. During the short incubation (five or ten minutes), a minimal amount of degradation of the thin film will occur. Resorption pit integrity remains intact with clearly visible borders. While the thin film may appear smoother, longer incubation times will increase staining intensity at the cost of dissolving the film completely. **Figure 1** shows rat bone marrow cells cultured for eight days (refer to the BD BioCoat Osteologic Bone Cell Culture System User Guide [F00B017]). This sample was stained for ten minutes. Results may vary depending on the cell type and culture conditions. A time-course study is recommended to achieve optimal results.

References

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