

## BD BioCoat™ Osteologic™ Bone Cell Culture System

### User Guide

The BD BioCoat™ Osteologic™ Bone Cell Culture System consists of submicron synthetic mineralized calcium phosphate thin films coated onto various culture vessels. This system can be used as an alternative method for compound screening for direct assessment of osteoclast and osteoblast activity *in vitro*.

BD BioCoat Osteologic Discs and MultiTest Slides provide highly uniform and consistent substrates that are easily used to characterize and measure osteoclast-mediated bone resorption. This assay platform provides an effective method for studying osteoclastogenesis.

For first time users, basic protocols for media preparation, cell culture growth, and cell removal are provided. For detailed information regarding the use of other cell or animal model systems, contact BD Biosciences Discovery Labware Technical Service Department at 800.343.2035.



### Media Preparation

#### Femur Wash Medium

- $\alpha$ -MEM (Gibco 12571-063) containing 15% FBS (Gibco 10437-028)
- 1 mg/ml Penicillin G (Sigma P-3032)
- 0.5 mg/ml Gentamycin (Gibco 15710-064)
- 3 mg/ml Fungizone (Gibco 15295-017)

#### Primary Culture Medium

- $\alpha$ -MEM (Gibco 12571-063) containing 15% FBS (Gibco 10437-028)
- 0.1 mg/ml Penicillin G (Sigma P-3032)
- 0.05 mg/ml Gentamycin (Gibco 15710-064)
- 300 ng/ml Fungizone (Gibco 15295-017)
- 0.28 mM L-Ascorbic Acid 2-Phosphate (Sigma A-8960)
- 10 nM Dexamethasone (Sigma D-2915)

#### Osteoclast Medium

- $\alpha$ -MEM (Gibco 12571-063) containing 15% FBS (Gibco 10437-028)
- 0.1 mg/ml Penicillin G (Sigma P-3032)
- 0.05 mg/ml Gentamycin (Gibco 15710-064)
- 300 ng/ml Fungizone (Gibco 15295-017)
- 0.28 mM L-Ascorbic Acid 2-Phosphate (Sigma A-8960)
- 10 mM  $\beta$ -Glycerophosphate (Sigma G-9891)

#### Osteoblast Medium

- $\alpha$ -MEM (Gibco 12571-063) containing 15% FBS (Gibco 10437-028)
- 300 ng/ml Fungizone (Gibco 15295-017)
- 0.28 mM L-Ascorbic Acid 2-Phosphate (Sigma A-8960)
- 10 nM Dexamethasone (Sigma D-2915)

### Preparation of Osteoclast Cultures

1. Excise femurs (following animal sacrifice) from young male (100-125 g) Wistar rats.
2. Pass femurs through 4 washes (30-40 ml each) of femur wash medium.
3. Perform final wash in 30-40 ml of osteoclast medium.
4. Remove epiphyses. Using a syringe flush marrow out with 10 ml of osteoclast medium from both ends of the shaft.
5. Mix cell suspension up and down.
6. Inoculate 1 ml onto each Disc or 0.25 ml/well of MultiTest Slide.
7. Incubate 37°C, 5% CO<sub>2</sub>, 100% humidity.
8. Change medium after 24 hours with osteoclast medium.
9. Refeed 3 times weekly as in Step 8 (*Note: When culturing at high cell density, it may be necessary to refeed the samples more frequently to maintain the culture environment at or near physiological pH. Since the BD Osteologic substrate can dissolve under acidic conditions, the culture medium should not go below pH 6.5.*)
10. Maintain osteoclast cultures for 8 to 10 days.
11. Wash cells with PBS and fix with glutaraldehyde for TRAP staining or remove cells to view total osteoclast resorption. TRAP Staining Protocol can be found in BD Biosciences Discovery Labware Technical Bulletin 445.

### Preparation of Osteoblast Cultures

1. Repeat Osteoclast Culture Steps 1-5.
2. Sieve cells through 100  $\mu$ m cell strainer into BD Falcon™ Conical Tube.
3. Spin cells for 10 minutes at 1,000 rpm.
4. Resuspend cells in Primary Culture medium (5 ml/femur).
5. Inoculate 75 cm<sup>2</sup> flasks with 5 ml of cells and additional 15 ml of primary culture medium.
6. Incubate 37°C, 5% CO<sub>2</sub>, 100% humidity.
7. Fluid change with 15 ml of primary culture medium per flask on Days 2 and 4.
8. Trypsinize and count cells on Day 6.
9. Inoculate 12,500 cells in 1 ml of osteoblast medium per Disc or 2,000 cells in 0.2 ml of osteoblast medium per well if using MultiTest Slide.
10. Incubate 37°C, 5% CO<sub>2</sub>, 100% humidity.
11. Change media every other day (*Note: When culturing at high cell density, it may be necessary to refeed the samples more frequently to maintain the culture environment at or near physiological pH. Since the BD Osteologic substrate can dissolve under acidic conditions, the culture medium should not go below pH 6.5.*)
12. Depending on experimental design, grow cells for 2 to 21 days.

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## Osteoclast Culture Cell Removal

### BD BioCoat™ Osteologic™ Discs

1. Remove medium and rinse cells with Milli-Q™ water.
2. Add 1 ml bleach solution (~6% NaOCl, ~5.2% NaCl) to each well.
3. Pipette up and down to dislodge cells.
4. Aspirate bleach after five minutes at room temperature.
5. Wash discs 3 times with ~2 ml distilled water. (*Note: It is important to wash the samples thoroughly to avoid salt crystal formation. After each wash step, carefully aspirate to ensure the complete removal of the wash solution. The dried sample should not contain salt deposits.*)
6. Allow to air dry and examine microscopically. If necessary, repeat Steps 2-5 to remove any remaining cells.

### BD BioCoat Osteologic MultiTest Slides

1. Remove medium.
2. To remove upper structure, grasp the projecting edge of the slide at the near corner. With the other hand pull upon the upper structure.
3. Place individual slides in petri dish or 4-slot plate. Rinse with Milli-Q water.
4. Cover slide with bleach and leave for five minutes.
5. Wash slides 3 times with distilled water (*Note: It is important to wash the samples thoroughly to avoid salt crystal formation. After each wash step, carefully aspirate to ensure the complete removal of the wash solution. The dried sample should not contain salt deposits.*)
6. Allow to air dry and examine microscopically.

## Microscopy

For light microscopy, use phase optics and/or dark-field with 20-100x magnification.

The use of high-quality optics will ensure the visualization of resorption lacunae.

*Note: Due to differences in imaging technique, cell cultures, and particularly the cell/thin film surface interface, images obtained using the BD BioCoat Osteologic products will appear different from those obtained using bone slices or dentine. Familiarity with this proven methodology is quickly gained with experience. It is not necessary to stain the films to visualize and/or measure resorption. If staining is performed, ensure that the pH is well controlled and does not fall below pH 6.5.*

If desired, bone resorption activity can be readily scanned by automated image analysis using the Millenium Biologix Microst™ Image Analyzer. For additional information on the Microst Screening Service, please contact Millenium Biologix at 613.389.6565. Alternatively, standard laboratory image analyzers such as Bioquant can be adapted for use with the BD BioCoat Osteologic Bone Cell Culture System.

BD BioCoat Osteologic products are compatible with SEM/TEM processing for more detailed investigations.

## Troubleshooting Hints

- Care must be taken when pipetting during cell seeding and refeeding, as well as during the cell removal and washing procedures. Scratch marks from pipette tips or tweezers will read as resorption pits on the Microst Image Analyzer.
- Incomplete rinsing of bleach can lead to inaccurate readings of resorption. Make sure discs/slides are thoroughly rinsed prior to analysis.
- When inoculating cells, direct the pipette over the center of the disc/well to ensure even cell seeding.

For additional staining methods and/or recommended instructions for chondrocyte culture, please request the following BD Biosciences Discovery Labware Technical Bulletins:

- **Technical Bulletin 444**  
von Kossa Staining of Osteoclast Resorption on BD BioCoat Osteologic Discs
- **Technical Bulletin 445**  
Tartrate Resistant Acid Phosphatase (TRAP) Staining of Osteoclasts on BD BioCoat Osteologic Discs and MultiTest Slides
- **Technical Bulletin 446**  
Chondrocyte Cell Culture and Tissue Engineering on BD BioCoat Osteologic Discs and BD™ 3D Calcium Phosphate Scaffolds

### For technical assistance, contact Technical Service at:

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