

Method of Measuring Pit Area

1. Plate Washing

- (1) Aspirate the culture medium completely from each well of the Bone Resorption Assay Plate and add 300 μ L/well of 5% sodium hypochlorite.
- (2) Incubate for five minutes at room temperature.
- (3) Wash the plate with tap water three times and with pure water once using a vat.
- (4) Dry the plate completely.

2. Observation of pits

- (1) Observe the pit formation in each well at 40x magnification. Pits will appear as individual or multiple clusters at the bottom of the well.
- (2) After focusing on the pits, turn off the light of the microscope.
- (3) Photograph pits in the well using a microscope with digital camera. The pits will be seen as black hole, and remaining calcium phosphate layer is as bright or white region.
- (4) Photograph five different regions in each well.

3. Measurement of pit area using Scion image software

- (1) Convert the all photo to the black and white image and enhance the contrast using Image software (Microsoft Office Picture Manager, Photoshop, etc).
- (2) Save the image as bitmaps. JPEG image cannot be analyzed by Scion Image Software.
- (3) Open an image of pits by Scion Image software.
- (4) Convert to a binary format image by using thresholding mode in order to distinguish between the pit area and background.
- (5) Measure pit area. Use “Show Results” to display the list of area measurement.
- (6) Measure all image under the same condition.
- (7) After finishing the measurement, “Export” and save the result.
- (8) Open the exported result file with an appropriate software and calculate the pit area in each well.

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