



# Standard Practice for Quantification of Calcium Deposits in Osteogenic Culture of Progenitor Cells Using Fluorescent Image Analysis<sup>1</sup>

This standard is issued under the fixed designation F2997; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon ( $\epsilon$ ) indicates an editorial change since the last revision or reapproval.

## 1. Scope

1.1 This practice defines a method for the estimation of calcium content at multiple time points in living cell cultures that have been cultured under conditions known to promote mineralization. The practice involves applying a fluorescent calcium chelating dye that binds to the calcium phosphate mineral crystals present in the live cultures followed by image analysis of fluorescence microscopy images of the stained cell cultures. Quantification of the positively stained areas provides a relative measure of the calcium content in the cell culture plate. A precise correlation between the image analysis parameters and calcium content is beyond the scope of this practice.

1.2 Calcium deposition in a secreted matrix is one of several features that characterize bone formation (*in vitro* and *in vivo*), and is therefore a parameter that may indicate bone formation and osteoblast function (i.e., osteoblastic differentiation). Calcium deposition may, however, be unrelated to osteoblast differentiation status if extensive cell death occurs in the cell cultures or if high amounts of osteogenic medium components that lead to artifactual calcium-based precipitates are used. Distinguishing between calcium deposition associated with osteoblast-produced mineralized matrix and that from pathological or artifactual deposition requires additional structural and chemical characterization of the mineralized matrix and biological characterization of the cell that is beyond the scope of this practice.

1.3 The parameters obtained by image analysis are expressed in relative fluorescence units or area percentage, e.g., fraction of coverage of the area analyzed.

1.4 *Units*—The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appro-*

*priate safety and health practices and determine the applicability of regulatory limitations prior to use.*

## 2. Referenced Documents

2.1 *ASTM Standards*:<sup>2</sup>

F2312 *Terminology Relating to Tissue Engineered Medical Products*

F2603 *Guide for Interpreting Images of Polymeric Tissue Scaffolds*

F2739 *Guide for Quantitating Cell Viability Within Biomaterial Scaffolds*

## 3. Terminology

3.1 Unless provided otherwise in 3.2, terminology shall be in conformance with F2312.

3.2 *Definitions*:

3.2.1 *mineralized matrix, n*—a calcium phosphate-containing substance produced by cells typically in the osteoblast, odontoblast, and calcifying chondrocyte lineages, which is composed of crystals of calcium phosphate and contains collagen Type I and other non-collagenous proteins.

3.2.2 *osteoblasts, n*—secretory mononuclear cells that will initiate the formation of a matrix containing characteristic proteins, such as collagen, and non-collagenous proteins such as bone sialoprotein and osteocalcin, that will mineralize in the presence of a calcium and phosphate source.

3.3 *Definitions of Terms Specific to This Standard*:

3.3.1 *calcium deposits, n*—a calcium phosphate-containing substance synthesized in cell cultures during mineralization assays; such as, osteoblast differentiation assays, that may have precipitated out of solution rather than being produced by the cells.

## 4. Summary of Practice

4.1 This practice consists of (1) fluorescently staining the calcium deposits in a cell culture using the non-toxic calcium-chelating dye xylenol orange, (2) collecting fluorescent microscopy images of the stained samples, (3) collecting images of

<sup>1</sup> This test method is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.43 on Cells and Tissue Engineered Constructs for TEMPs.

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<sup>2</sup> For referenced ASTM standards, visit the ASTM website, [www.astm.org](http://www.astm.org), or contact ASTM Customer Service at [service@astm.org](mailto:service@astm.org). For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.