



Standard Guide for Quantifying Cell Viability within Biomaterial Scaffolds¹

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1. Scope

1.1 This guide is a resource of cell viability test methods that can be used to assess the number and distribution of viable and non-viable cells within porous and non-porous, hard or soft biomaterial scaffolds, such as those used in tissue-engineered medical products (TEMPs).

1.2 In addition to providing a compendium of available techniques, this guide describes materials-specific interactions with the cell assays that can interfere with accurate cell viability analysis, and includes guidance on how to avoid, and/or account for, scaffold material/cell viability assay interactions.

1.3 These methods can be used for 3-D scaffolds containing cells that have been cultured *in vitro* or for scaffold/cell constructs that are retrieved after implantation in living organisms.

1.4 This guide does not propose acceptance criteria based on the application of cell viability test methods.

1.5 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

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

2. Referenced Documents

2.1 *ASTM Standards:*²

F748 Practice for Selecting Generic Biological Test Methods for Materials and Devices

F2149 Test Method for Automated Analyses of Cells—the

Electrical Sensing Zone Method of Enumerating and Sizing Single Cell Suspensions
F2315 Guide for Immobilization or Encapsulation of Living Cells or Tissue in Alginate Gels
F2998 Guide for Using Fluorescence Microscopy to Quantify the Spread Area of Fixed Cells

3. Terminology

3.1 *Definitions:*

3.1.1 *non-viable cell, n*—a cell not meeting one or more of the criteria for a viable cell.

3.1.2 *viable cell, n*—a cell capable of metabolic activity that is structurally intact with a functioning cell membrane.

4. Summary of Guide

4.1 It is the intent of this guide to provide a compendium of the commonly used methods for quantifying the number and distribution of viable and non-viable cells within, or on, a biomaterial scaffold, because cell viability is an important parameter of tissue-engineered products used to regenerate or repair lost or diseased tissue. The methods can be applied to cells residing within an intact 3-D scaffold or matrix (that is, non-destructive methods) or to cells that have been removed from the scaffold or matrix (that is, destructive methods). It should be noted that not all cells require a scaffold and some cell types, such as hematopoietic cells, cannot be cultured or grown on an adherent surface.

4.2 Most of the methods originate from analysis of cell number on 2-D surfaces, but have been adapted for the analysis of cells within 3-D constructs that are typically used in regenerative medicine approaches. The mechanisms and the sensitivity of the assays are discussed. The limitations of the assays due to using standard curves generated from cells on 2-D surfaces are described in this document. In addition, the ways in which the biomaterial scaffold itself can affect the viability assays are described.

4.3 This guide describes test methods which, when used together, may enable accurate measure of the number and distribution of viable and non-viable cells. Different viability assays have different measurands, which means that the results from different assays may not correlate with one another. For instance, cell membrane integrity tests and cell metabolic tests

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² For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.