CellTiter-Fluor™ Cell Viability Assay

96-Well Plate Protocol for Cell Plating and Viability Measurement

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Assay Overview

The CellTiter-Fluor™ Cell Viability Assay is a nonlytic, fluorescence-based assay that measures the relative number of live cells by detecting a conserved protease activity within intact viable cells. The fluorogenic substrate (GF-AFC) enters living cells where it is cleaved by the live-cell protease to generate a fluorescent signal proportional to the number of viable cells.

SAFETY & IMPORTANT NOTES

- Always wear appropriate PPE: lab coat, gloves, eye protection
- Work in a biological safety cabinet when handling cells
- DMSO is present in the substrate - handle with care
- Shield plates from ambient light during incubation
- Do not incubate longer than 3 hours

⏱ Time Required

- Plate setup: 30-60 min
- Treatment period: Variable
- Assay incubation: 1.5-2 hours
- Reading: 5-10 min

🔬 Key Features

- Nonlytic assay
- Single reagent addition
- High sensitivity

• Compatible with multiplexing

📊 Applications

• Drug cytotoxicity screening
• Cell proliferation assays
• Compound testing
• Dose-response curves

⚠️ Critical: Edge Effect Prevention

Always fill the perimeter wells of the 96-well plate with PBS only (no cells). This prevents edge effects caused by differential evaporation and temperature gradients that can affect the outer wells. Only the inner 60 wells (rows B-G, columns 2-11) should contain cells and experimental conditions.

Assay Principle

1. **Substrate Entry:** Cell-permeant GF-AFC substrate crosses the membrane of viable cells
2. **Enzymatic Cleavage:** Live-cell protease cleaves the substrate to release fluorescent AFC
3. **Signal Generation:** Fluorescence intensity is proportional to the number of viable cells
4. **Dead Cell Exclusion:** Dead cells with compromised membranes lose protease activity and don't generate signal

Detection Parameters:

• **Excitation:** 380-400 nm
• **Emission:** 505 nm
• **Readout:** Fluorescence (RFU - Relative Fluorescence Units)

Protocol adapted from Promega CellTiter-Fluor™ Technical Bulletin TB371

Lab-specific modifications: 1:1000 dilution, 25 µL/well, 1.5-2 hr incubation

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