

## CellTiter-Glo® 3D Cell Viability Assay

### Robust, Validated ATP Viability Assay for 3D Cell Culture

Assays for measuring viability in monolayer (2D) cell cultures are not sufficiently robust for 3D cell culture samples, especially those with larger microtissues, because 2D cell culture assays lack the ability to penetrate the layers of cells present in 3D cell cultures. The CellTiter-Glo® 3D Cell Viability Assay provides a homogeneous method to determine the number of viable cells in 3D cell cultures. The CellTiter-Glo® 3D Cell Viability Assay is based on quantitation of ATP, which signals the presence of metabolically active cells. The ready-to-use reagent is based on the original CellTiter-Glo® Assay chemistry, with improved lytic capacity for 3D cell cultures. The assay can be used with various multiwell formats (96-well and regular or low-volume 384-well).

#### Advantages

- **Robust Penetration into Microtissue:** Improved lytic capacity allows use over a broad range of microtissue sizes compared to other viability assay methods.
- **Ready-to-Use Reagent:** Simply thaw, equilibrate and “add-mix-incubate-measure”. Convenient for HTS applications.
- **Fast:** Data can be recorded in 30 minutes or less after adding reagent, quicker than when using colorimetric or fluorometric viability assays.
- **Superior Sensitivity:** The signal-to-background ratio of this assay applied to microtissues is much greater than that of standard colorimetric and fluorometric assays.
- **Glow-Type Signal:** Stable luminescent signal half-life >3 hours, depending on cell type and culture medium used, allows batch mode or consecutive processing of multiple plates.

#### Improved ATP Recovery from Larger Spheroids

| Diameter of Spheroid (µm) | ATP Recovered (pmol/microtissue) |                         |       |
|---------------------------|----------------------------------|-------------------------|-------|
|                           | Classic CellTiter-Glo® Assay     | CellTiter-Glo® 3D Assay | Ratio |
| 188                       | 16 ± 4                           | 17 ± 4                  | 1.10  |
| 386                       | 79 ± 3                           | 94 ± 11                 | 1.19  |
| 459                       | 103 ± 2                          | 126 ± 11                | 1.22  |
| 565                       | 127 ± 3                          | 178 ± 17                | 1.40  |

**Table 1. CellTiter-Glo® 3D Cell Viability Assay has improved ATP detection from larger spheroids compared to the classic CellTiter-Glo® Assay.**

Different amounts of HCT116 cells (RPMI medium +10% FBS) were seeded in InSphero GravityPLUS™ 96-well hanging-drop platform and grown for 4 days until microtissues of the indicated sizes formed. Microtissues were assayed by adding an equal volume of reagent to medium volume, shaking for 5 minutes, and recording luminescence with a GloMax® luminometer after 30 minutes incubation at room temperature. The amount of ATP detected was determined by comparison with ATP standards.

#### Assay Principle

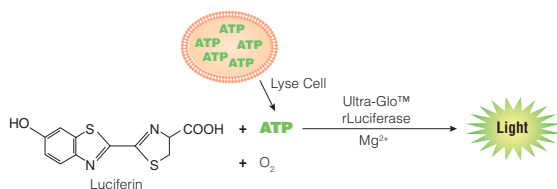


**Figure 1.** Viable cells are the source of ATP in the luciferase reaction. The CellTiter-Glo® 3D Reagent contains Ultra-Glo™ Recombinant Luciferase and luciferin and is added directly to cells in culture. The reagent simultaneously lyses cells to release ATP while protecting ATP from degradation. The ATP is used in the luciferase reaction to produce light. The amount of light produced is directly proportional to the number of viable cells in the microtissue.

#### Simple, Homogeneous Protocol

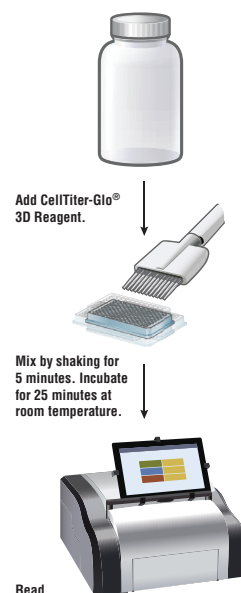


**Figure 2.** CellTiter-Glo® 3D Cell Viability Assay protocol.

## Improved ATP Detection Compared to Competitor ATP Assay

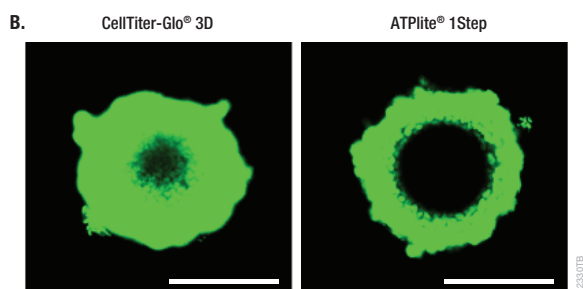
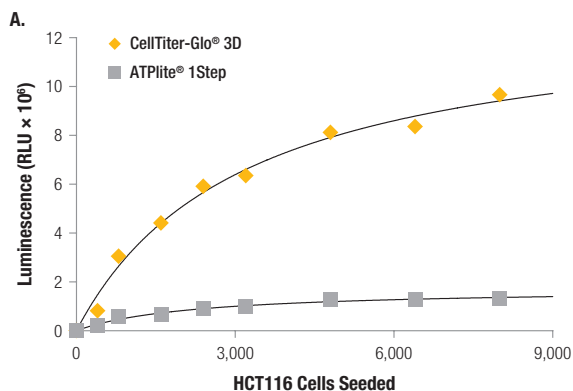


**Figure 3. CellTiter-Glo® 3D Reagent shows improved lytic capacity compared to ATPlite® 1Step Reagent.** HCT116 colon cancer spheroids were generated as described in Table 1. **Panel A.** An equivalent volume of CellTiter-Glo® 3D or ATPlite® 1step reagent was added to all samples, samples were shaken for 5 minutes, and luminescence was recorded at 30 minutes. **Panel B.** A 2X concentration of CellTox™ Green Dye (cell-impermeable DNA binding dye) was added to both CellTiter-Glo® 3D Reagent (left) and ATPlite® 1step reagent (right) prior to sample addition. After 5 minutes of shaking and a 30-minute incubation at room temperature, confocal laser fluorescent microscopy images were taken. Green staining indicates cell lysis. The spheroids in **Panel B** are 300µm in diameter, and the bars in each image represent a distance of 200µm.

For more information about the CellTiter-Glo® 3D Cell Viability Assay, visit: [promega.com/CellTiterGlo3D](http://promega.com/CellTiterGlo3D)

## Ordering Information

| Product                                | Size      | Cat.# |
|----------------------------------------|-----------|-------|
| CellTiter-Glo® 3D Cell Viability Assay | 10ml      | G9681 |
|                                        | 10 × 10ml | G9682 |
|                                        | 100ml     | G9683 |

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## Superior Sensitivity Compared to Competitor Assays

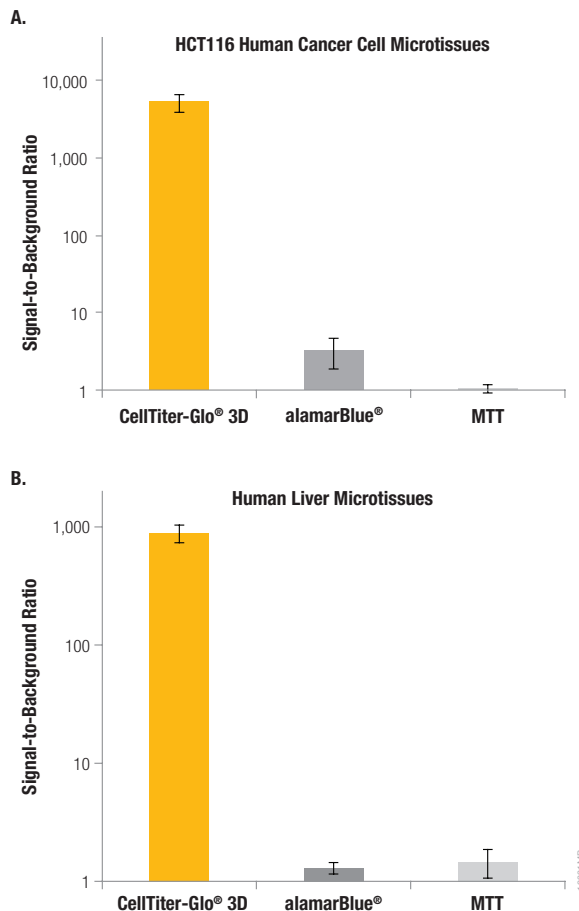


**Figure 4. CellTiter-Glo® 3D Assay sensitivity compared to other fluorescent and colorimetric viability assays.** **Panel A.** HCT116 colon cancer cells were seeded into an InSphero GravityPLUS™ 96-well hanging-drop platform and grown for 4 days to generate ~340µm spheroids. **Panel B.** Insight™ human liver microtissues (~250µm) were provided by InSphero. All microtissues were assayed using the CellTiter-Glo® 3D, alamarBlue® and MTT assays according to manufacturers' protocols. The total assay times for the CellTiter-Glo® 3D, alamarBlue® and MTT assays were 30 minutes, 3 hours, and 8 hours, respectively.

