TECHNICAL MANUAL

CellTiter-Glo® 3D Cell Viability Assay

Instructions for Use of Products
G9681, G9682 and G9683

Revised 11/15
TM412

CellTiter-Glo® 3D Cell Viability Assay

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1. Description

The CellTiter-Glo® 3D Cell Viability Assay [a–c] is a homogeneous method to determine the number of viable cells in 3D cell culture based on quantitation of the ATP present, which is a marker for the presence of metabolically active cells. This ready-to-use reagent is based on the original CellTiter-Glo® Luminescent Cell Viability Assay chemistry and eliminates the need to combine buffer with lyophilized substrate when preparing reagent. The CellTiter-Glo® 3D Cell Viability Assay is formulated with more robust lytic capacity and is designed for use with microtissues produced in 3D cell culture, although it is similar in performance to the classic reagent when assaying monolayers of cells produced in 2D cell culture. This assay is compatible with multiwell-plate formats, making it ideal for automated high-throughput screening (HTS) using cell proliferation and cytotoxicity assays. The homogeneous assay procedure (Figure 1) involves addition of a single reagent (CellTiter-Glo® 3D Reagent) directly to cells cultured in serum-supplemented medium. Cell washing, removal of medium and multiple pipetting steps are not required.

1. Description (continued)

The luciferase reaction for this assay is shown in Figure 2. The homogeneous "add-mix-measure" format results in cell lysis and generation of a luminescent signal proportional to the amount of ATP present (Figure 3, Panel A). The amount of ATP is directly proportional to the number of viable cells present in culture (depending on culture conditions) in agreement with previous reports (1). The linear correlation can be easily demonstrated with cells in 2D cell culture (Figure 3, Panel B). For 3D cell culture, the relationship between seeded cell number and luminescent output after several days in culture is often curvilinear due to the effects of contact inhibition on cell proliferation, as well as reduced metabolic activity and/or necrosis in the central region of large microtissues (Figure 3, Panel C). However, a linear correlation can be shown between the luminescent signal of the CellTiter-Glo® 3D Assay and the fluorescent signal of a DNA-binding dye, CellTox™ Green Dye (Figure 3, Panel D). The fact that the amount of ATP is proportional to the amount of DNA illustrates that the CellTiter-Glo® 3D Cell Viability Assay effectively reports the number of viable cells in 3D cell culture. The CellTiter-Glo® 3D Cell Viability Assay relies on the properties of a proprietary thermostable luciferase (Ultra-Glo™ Recombinant Luciferase), which generates a stable "glow-type" luminescent signal and improves performance across a wide range of assay conditions (Section 4.A). The half-life of the luminescent signal resulting from this reaction is greater than three hours. This extended half-life eliminates the need for reagent injectors and provides flexibility for continuous or batch-mode processing of multiple plates.

Assay Advantages

- **Robust penetration into microtissues:** Improved lytic capacity allows use over a broad range of microtissue sizes compared to other viability assay methods.
- **Ready-to-use reagent:** No mixing of components required; simply thaw, equilibrate to room temperature and "add-mix-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.
- **Flexible:** The assay can be used with various multiwell formats (96-well and regular or low-volume 384-well). Data can be recorded by luminometer, CCD camera or other imaging devices capable of reading luminescence in multiwell plates.
- **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.

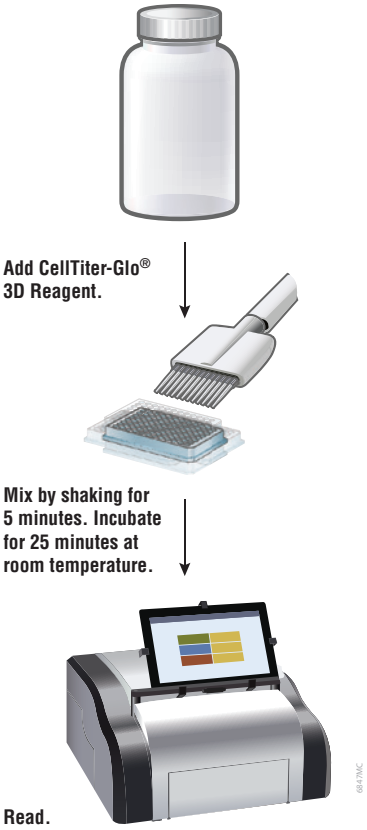

Figure 1. Overview of the CellTiter-Glo® 3D Cell Viability Assay protocol.

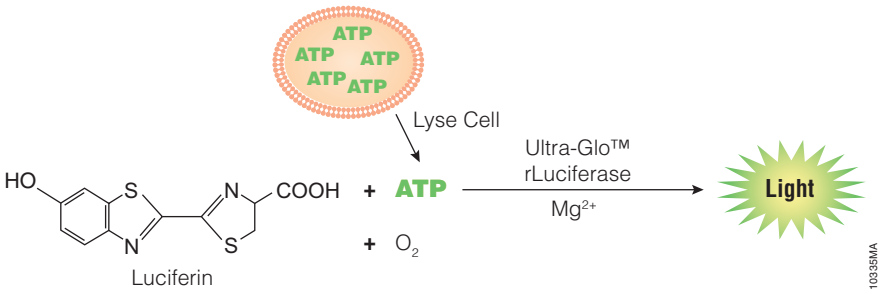

Figure 2. CellTiter-Glo® 3D Cell Viability Assay principle.

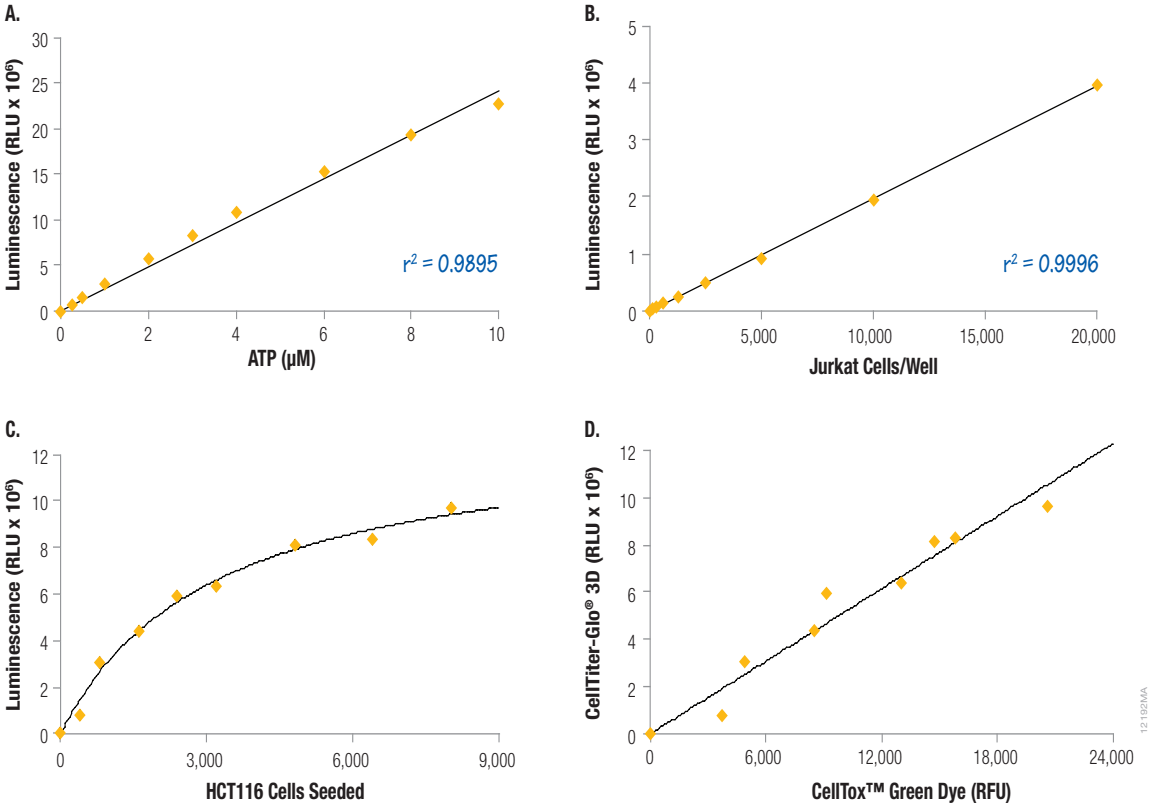

Figure 3. ATP concentration and/or cell number correlates with luminescence output. Panel A. Different concentrations of ATP in water were plated at 100µl. **Panel B.** Dilutions of Jurkat cells were made in RPMI 1640 with 10% FBS and plated at 100µl of cells per well. **Panels C and D.** HCT116 colon cancer spheroids were generated by seeding cells into a 96-well GravityPLUS™ hanging-drop plate (InSphero AG); cells were grown for 4 days. All samples were added or transferred to a standard white assay plate and an equivalent volume of CellTiter-Glo® 3D Reagent was added. For cells in 2D culture, the plate was shaken for 2 minutes and luminescence was recorded 10 minutes after reagent addition. For microtissues in 3D culture, the plate was shaken for 5 minutes and luminescence was recorded 30 minutes after reagent addition. For the 3D cell culture samples, a 2X concentration of CellTox™ Green Dye was added to the CellTiter-Glo® 3D Reagent prior to sample addition.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
CellTiter-Glo® 3D Cell Viability Assay	**10ml**	**G9681**

For Research Use Only. Not for use in diagnostic procedures. G9681 is sufficient for 100 assays at 100µl/assay in 96-well plates or 400 assays at 25µl/assay in 384-well plates.

PRODUCT	SIZE	CAT.#
CellTiter-Glo® 3D Cell Viability Assay	**10 × 10ml**	**G9682**

For Research Use Only. Not for use in diagnostic procedures. G9682 is sufficient for 1,000 assays at 100µl/assay in 96-well plates or 4,000 assays at 25µl/assay in 384-well plates.

PRODUCT	SIZE	CAT.#
CellTiter-Glo® 3D Cell Viability Assay	**100ml**	**G9683**

For Research Use Only. Not for use in diagnostic procedures. G9683 is sufficient for 1,000 assays at 100µl/assay in 96-well plates or 4,000 assays at 25µl/assay in 384-well plates.

Storage Conditions: The CellTiter-Glo® 3D Cell Viability Assay is shipped frozen and can be stored at –30 to –10°C through the expiration date on the kit label. The CellTiter-Glo® 3D Reagent will maintain >90% activity upon storage at 4°C for 3.5 days or at room temperature (22–25°C) for 12 hours. The reagent can withstand three additional freeze-thaw cycles after the first thaw with no significant loss of activity. We do not recommend dispensing the CellTiter-Glo® 3D Reagent into aliquots due to the risk of ATP contamination.

3. Performing the CellTiter-Glo® 3D Cell Viability Assay

Materials to Be Supplied by the User

- 22°C water bath
- opaque-walled multiwell plates suitable for 3D cell culture
- multichannel pipet or automated pipetting station
- device (plate shaker) for mixing multiwell plates
- luminometer, CCD camera or imaging device capable of reading luminescence in multiwell plates
- **optional:** ATP for use in generating a standard curve (see Section 3.C; Promega Cat.# P1132, Sigma Cat.# A7699 or GE Healthcare Cat.# 27-1006)

Promega

3.A. Reagent Preparation

1. Thaw the CellTiter-Glo® 3D Reagent at 4°C overnight.

Do not thaw the reagent by placing the frozen bottle directly into a water bath as the bottle may break.

2. Equilibrate the CellTiter-Glo® 3D Reagent to room temperature by placing the reagent in a 22°C water bath prior to use for approximately 30 minutes.

3. Mix gently by inverting the contents to obtain a homogeneous solution.

   Note: Use caution when removing the seal of the CellTiter-Glo® 3D Reagent bottle to avoid introducing ATP contamination.

3.B. Protocol for the Cell Viability Assay

1. Prepare opaque-walled multiwell plates with microtissues in culture medium. Sample volumes and microtissue properties (e.g., size, number, days in culture, etc.) should be optimized for experimental conditions. For example, the total ATP content of the samples should be kept below the 10µM limit of the assay linearity (see Section 4.B). Multiwell plates must be compatible with the luminometer used.

2. Add test compound to experimental wells, and incubate according to your culture protocol.

   Be sure that the volume of the sample plus test compounds is low enough to allow addition of an equal volume of reagent, and subsequent mixing without well-to-well contamination.

Equilibrate the plate and its contents to room temperature (22–25°C) for approximately 30 minutes.

4. Add a volume of CellTiter-Glo® 3D Reagent equal to the volume of cell culture medium present in each well (e.g., for a 96-well plate, add 100µl of CellTiter-Glo® 3D Reagent to 100µl of medium containing cells).

5. Mix the contents vigorously for 5 minutes to induce cell lysis.

   Note: Mixing is very important for effective extraction of ATP from 3D microtissues. See the Appendix, Section 4, for more information on mixing.

6. Allow the plate to incubate at room temperature for an additional 25 minutes to stabilize the luminescent signal.

7. Record luminescence.

   Notes:

   Detection instrument settings depend on the manufacturer. Use an integration time of 0.25–1 second per well as a guideline.

   An uneven luminescent signal within plates can be caused by temperature gradients, uneven seeding of cells or edge effects in multiwell plates.

3.C. Protocol for Generating an ATP Standard Curve (optional)

It is a good practice to generate a standard curve using the same plate on which samples are assayed. Because of endogenous ATPases found in serum, the ATP standard curve should be generated immediately prior to adding the CellTiter-Glo® 3D Reagent. If the amount of ATPases is sufficiently high, it may be necessary to omit serum from the medium used to generate the standard curve. We recommend ATP disodium salt (Promega Cat.# P1132, Sigma Cat.# A7699 or GE Healthcare Cat.# 27-1006).

1. Prepare 10µM ATP in culture medium (100µl of 10µM ATP solution contains 10^{-9} moles of ATP).
2. Dilute the 10µM solution to produce a range of ATP concentrations in culture medium. For monolayers in 2D cell culture, we recommend a range of 10µM to 10nM of ATP. For microtissues in 3D cell culture, there is typically a significant amount of ATP present, so the necessary range of ATP concentrations can be skewed toward the higher end.
3. Prepare a multiwell plate with varying concentrations of ATP standard in 100µl of medium (25µl for a 384-well plate). In order to compare standards with samples, the same volume should be used for both.
4. Add a volume of CellTiter-Glo® 3D Reagent equal to the volume of ATP standard present in each well.
5. Mix contents for 5 minutes on an orbital shaker.
6. Allow the plate to incubate at room temperature for an additional 25 minutes.
7. Record luminescence.
8. Compare luminescence of samples to luminescence of standards to determine ATP detected by the CellTiter-Glo® 3D Reagent in samples.

4. Appendix

4.A. Overview of the CellTiter-Glo® 3D Cell ViabilityAssay

The CellTiter-Glo® 3D Cell Viability Assay takes advantage of the properties of a proprietary thermostable luciferase, Ultra-Glo™ Recombinant Luciferase, to enable reaction conditions that generate a stable "glow-type" luminescent signal while simultaneously inhibiting endogenous enzymes released during cell lysis (e.g., ATPases). Release of ATPases will interfere with accurate ATP measurement. Historically, firefly luciferase purified from *Photinus pyralis* (LucPpy) has been used in reagents for ATP assays (1–5). However, LucPpy has only moderate stability in vitro and is sensitive to factors in the chemical environment, including pH and detergents, which can limit its usefulness for developing a robust homogeneous ATP assay.

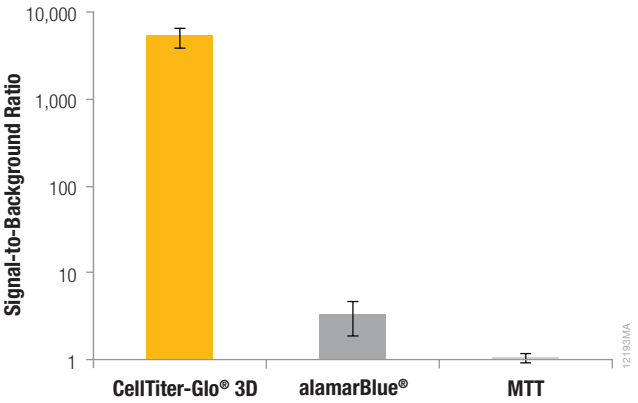

Figure 4. Sensitivity comparison of different viability assays applied to 3D microtissues. Four hundred HCT116 colon cancer cells were seeded into a 96-well GravityPLUS™ hanging-drop plate (InSphero AG) and incubated for 4 days. Spheroids (~340µm) were assayed according to each of the assay manufacturers' protocols. The total assay times for the CellTiter-Glo® 3D, alamarBlue® and MTT assays were 30 minutes, 3 hours and 8 hours (4 hours with MTT alone and an additional 4 hours with acidified SDS for solubilization), respectively.

We successfully developed a stable form of luciferase based on the gene from a different firefly, *Photuris pennsylvanica* (LucPpe2) by selecting characteristics that improved performance in ATP assays. The unique characteristics of this mutant (LucPpe2m), Ultra-Glo™ Recombinant Luciferase, enabled design of a homogeneous single-reagent-addition approach for performing ATP assays on cultured cells. Properties of the CellTiter-Glo® 3D Cell Viability Assay overcome problems caused by factors such as ATPases that interfere with the measurement of ATP in cell extracts. Moreover, the enhanced stability of Ultra-Glo™ Recombinant Luciferase allows the use of higher detergent concentrations to achieve the greater lytic capacity required for 3D microtissues. The reagent is robust and provides a sensitive and stable luminescent output.

Sensitivity and Linearity: The ATP-based detection of cells is more sensitive than other methods (6–8). This has been well established for cells in 2D cell culture, but it is also true for cells in 3D cell culture (Figure 4). There is a linear relationship between luminescent signal and cell number and/or ATP in different plate formats (Figure 3), and this relationship is maintained regardless of when the luminescence is recorded within the half-life of the assay (data not shown).

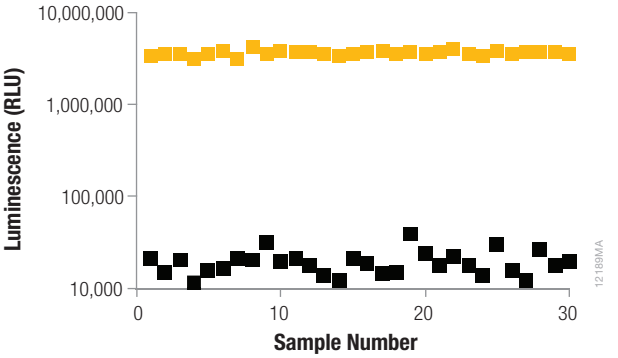

Figure 5. Z´-factor experiment with 3D microtissues. Four hundred HCT116 colon cancer cells were seeded into each of 60 wells of a 96-well GravityPLUS™ hanging-drop plate (InSphero AG) and incubated for 4 days to form 60 spheroids (~350µm in diameter). Half of these spheroids were treated with 100µM panobinostat (black squares), and the other half were treated with vehicle (1% DMSO, orange squares). After 48 hours, all samples were assayed with the CellTiter-Glo® 3D Reagent.

Automation: Z´-factor is a measure of assay precision and a metric commonly used to evaluate the suitability of an assay for screening applications (9). Any value greater than 0.5 is typically deemed acceptable, and the quality of the assay increases as the Z´-factor approaches 1. To demonstrate the precision of the CellTiter-Glo® 3D Cell Viability Assay, the reagent was used to assay an entire 384-well plate, with half of the wells containing 600 Jurkat cells as the positive control and the other half containing medium only as the negative control (each well at a volume of 8µl). This yielded a Z´-factor of 0.83 (data not shown); hence, the precision of the CellTiter-Glo® 3D Cell Viability Assay is excellent. To demonstrate its precision in an actual 3D cell culture experiment, 400 HCT116 colon cancer cells were seeded into 60 wells of a 96-well GravityPLUS™ hanging-drop plate (InSphero AG) and incubated for 4 days to form 60 spheroids (~350µm in diameter). Half of these spheroids were treated with 100µM panobinostat as the negative control, and the other half were treated with vehicle (1% DMSO) as the positive control. After 48 hours, all samples were assayed with the CellTiter-Glo® 3D Reagent, and the Z´-factor was calculated to be 0.81 (Figure 5).

Speed: The homogeneous procedure for measuring ATP using the CellTiter-Glo® 3D Cell Viability Assay is faster than other commonly used methods for measuring the number of viable cells (such as MTT, alamarBlue® or Calcein-AM) that require prolonged incubation steps to enable the cellular metabolic machinery to convert indicator molecules into a detectable signal.

4.B. Important Considerations for 3D Microtissues

Among the ever growing number of 3D cell culture models, we have tested a small representative subset including scaffold-free, biological scaffold and synthetic scaffold models. The CellTiter-Glo® 3D Cell Viability Assay has been effective with microtissues produced via hanging-drop plates (InSphero AG), ultra-low attachment plates, Matrigel™-coated plates, agarose-coated plates, cultures suspended in methylcellulose and Alvetex® plates (Reinnervate Ltd). We expect effective performance in similar models, but we cannot guarantee performance in all models. Moreover, we cannot guarantee performance of the CellTiter-Glo® 3D Reagent with actual biological tissue samples.

Cellular ATP Content: Different cell types produce different amounts of ATP, and values reported for the ATP level in a particular cell type vary considerably (1,4,10–11). Moreover, there is typically a gradient of decreasing ATP concentration from the viable cell layer on the exterior to the nonviable cells at the center of microtissues, and the relative change in this gradient can vary considerably with cell type (12). It is possible with some 3D cell culture methods to generate large amounts of biomass in a single well that can impact the performance of the assay. This typically occurs around 10µM ATP, which is the upper limit of the assay linearity. Performing an ATP standard curve (Section 3.C) can confirm whether the luminescence produced by a given 3D cell culture method is under the 10µM limit. If the biomass and/or ATP is in excess, we recommend that you either use smaller microtissue samples (if possible), or dilute the mixture of sample and reagent before recording luminescence (Section 4.C). For example, after adding CellTiter-Glo® 3D Reagent to a sample with high biomass or ATP and shaking for 5 minutes, this mixture can be diluted tenfold into water. An aliquot of the dilution should then be mixed 1:1 with CellTiter-Glo® 3D Reagent prior to recording luminescence; it is important to add reagent soon after dilution to avoid any loss of ATP. The initial reagent addition is necessary to maximize lysis and prevent ATP degradation, while the subsequent dilution will reduce the concentration of ATP and biomass present, yet maintain the important reagent properties of this assay.

Mixing: Optimum assay performance is achieved when the CellTiter-Glo® 3D Reagent is completely mixed with the cultured cells. Inefficient mixing can result in under-reporting of the amount of ATP present and may yield inaccurate results. We recommend 5 minutes of vigorous shaking and a total of 30 minutes between reagent addition and recording of luminescence; however, less shaking or a shorter read time may be sufficient depending on the cell type and 3D cell culture method used. Vigorous pipetting (e.g., pipetting sample 8 times) can be used instead, but it typically produces greater signal variability than shaking. Other parameters related to reagent mixing include the force of delivery of the CellTiter-Glo® 3D Reagent, sample volume and dimensions of the well. All of these factors may affect assay performance. The degree of reagent mixing required may be affected by the method used to add CellTiter-Glo® 3D Reagent to the assay plates. Automated pipetting devices that use a greater or lesser force of fluid delivery may affect the degree of subsequent mixing required. Complete reagent mixing in 96-well plates can be achieved using orbital plate shaking devices built into many luminometers and the recommended 5 minute shaking time. Special electromagnetic shaking devices using a radius smaller than the well diameter may be required to efficiently mix the contents of 384-well plates (13). The depth of medium and geometry of the multiwell plates may have an effect on mixing efficiency. We recommend that you consider these factors when performing the assay to determine how much mixing may be necessary for your application. Optimization may be required for your particular system.

4.B. Important Considerations for 3D Microtissues (continued)

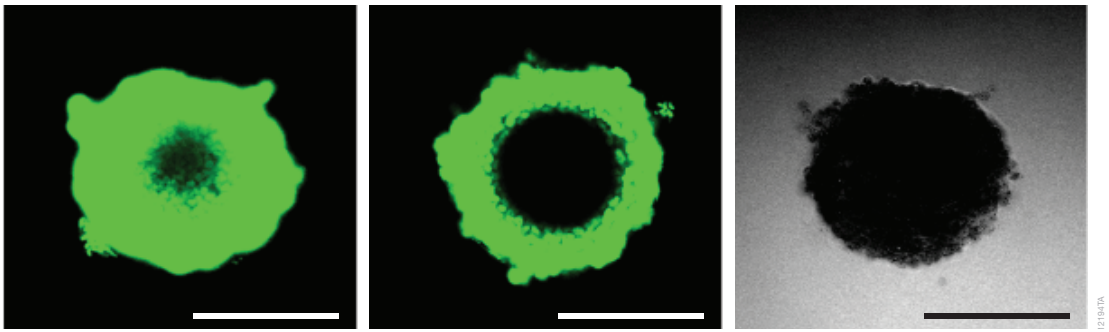

Figure 6. Reagent penetration and spheroid integrity. Four hundred HCT116 colon cancer cells were seeded into a 96-well GravityPLUS™ hanging-drop plate (InSphero AG) and incubated for 4 days. CellTox™ Green Dye was added to the CellTiter-Glo® 3D Reagent (left and right images) and a competitor's ATP detection assay for cell viability (center image) prior to assay. After adding an equal volume of reagent to each sample, they were shaken for 5 minutes, and the images above were taken at 30 minutes after reagent addition. The confocal laser fluorescent microscopy images show greater penetration of the CellTiter-Glo® 3D Reagent (left vs. center image), while the DIC (differential interference contrast) image (right) shows that the CellTiter-Glo® 3D Reagent is not destroying the overall microtissue structure. The bars in each image represent a distance of 200µm.

Note: Upon visual inspection of 3D microtissues treated with the CellTiter-Glo® 3D Reagent, complete loss of apparent microtissue morphology is not always evident even though lysis of individual cells is essentially complete. For example, cell spheroids treated with the CellTiter-Glo® 3D Reagent may slightly swell or some peripheral cells may slough off, but the overall integrity of the sphere may appear to be intact due to significant extracellular matrix structures (right panel, Figure 6). However, visualization of spheroid cell lysis, using a membrane-impermeant, fluorogenic DNA-binding dye in conjunction with confocal laser fluorescent microscopy reveals excellent cell lysis, especially in comparison to other bioluminescent cell viability assays (left and center panels, Figure 6). Moreover, by comparing the amount of ATP extracted by the CellTiter-Glo® 3D Reagent with the amount obtained by acid extraction (see Section 4.C), the CellTiter-Glo® 3D Reagent generally recovers >70% of the ATP present. The reagent penetration and ATP recovery decrease as microtissues become large, but for small to moderate size spheroids (~350µm or less), penetration and recovery are excellent.

4.C. Protocol for Assessing ATP Recovery

This is an example of an acid extraction protocol for determination of the total ATP present in a sample. In order to calculate the percentage of ATP recovered by the CellTiter-Glo® 3D Reagent from a specific sample, the amount of ATP detected by the CellTiter-Glo® 3D Reagent must be determined with a separate ATP standard curve (see Section 3.C). The ratio of ATP detected by the CellTiter-Glo® 3D Reagent versus the total ATP present gives the percent of ATP recovered.

1. Prepare an acid solution of 10% trichloroacetic acid and 4mM CDTA (diaminocyclohexane tetraacetic acid) in water.
2. Prepare opaque-walled multiwell plates with microtissues in culture medium (as in Section 3.B) and a range of ATP concentrations in culture medium (as inSection 3.C). All samples should be at the same volume.
3. Add a volume of acid solution equal to the volume of cell culture medium present in each well for both the samples containing microtissues and ATP standards.
4. Mix the contents vigorously for 5 minutes on an orbital shaker, and allow the plate to incubate at room temperature for an additional 15 minutes.
5. Transfer half of the sample to a separate container and dilute tenfold with water. For example, for a 100µl sample mixed with 100µl of acid solution, transfer 100µl and dilute with 900µl water. Mix samples repeatedly before and after dilution to ensure homogeneity. Theoretically, any amount of the sample can be transferred for dilution, but we have found that the variability between identical samples is less when larger fractions are transferred.
6. Transfer a volume of the diluted sample to a fresh assay well, and add an equal volume of CellTiter-Glo® 3D Reagent.
7. Mix contents for 2 minutes on an orbital shaker.
8. Allow the plate to incubate at room temperature for 10 minutes to stabilize the luminescent signal.
9. Record luminescence.
10. Compare luminescence of samples to luminescence of standards to determine total ATP present in samples.

4.D. Additional Considerations

Temperature: The intensity and rate of decay of the luminescent signal from the CellTiter-Glo® 3D Cell Viability Assay depends on the luciferase reaction rate. Environmental factors that affect the luciferase reaction rate will change the intensity of light output and stability of the luminescent signal. Temperature is one factor that affects the rate of this enzymatic assay and thus the light output. For consistent results, equilibrate assay plates to a constant temperature before performing the assay. Transferring eukaryotic cells from 37°C to room temperature has little effect on ATP content. For batch mode processing of multiple assay plates, take precautions to ensure complete temperature equilibration. Plates removed from a 37°C incubator and placed in tall stacks at room temperature will require a longer equilibration time than plates arranged in a single layer. Insufficient temperature equilibration may result in a temperature gradient between wells in the center and on the edge of the plates. The temperature gradient pattern also may depend on the position of the plate in the stack.

Chemicals: The chemical environment of the luciferase reaction affects the enzymatic rate and thus luminescence intensity. Differences in luminescence intensity have been observed using different types of culture media and sera. The presence of phenol red in culture medium should have little effect on luminescence output. Solvents for the test compounds may interfere with the luciferase reaction and thus light output. To test for luciferase inhibition, assemble two reactions, one with equal volumes of CellTiter-Glo® 3D Reagent and 1µM ATP, and a second reaction with equal volumes of CellTiter-Glo® 3D Reagent and 1µM ATP plus the test compound. Incubate reactions for 10 minutes at 22–25°C, then measure luminescence. A decrease in luminescence in the presence of test compound suggests luciferase inhibition.

Plate Recommendations: We recommend using standard opaque-walled multiwell plates suitable for luminescence measurements. Opaque-walled plates with clear bottoms, which allow microscopic visualization of cells, also may be used; however, assays in these plates will have diminished signal intensity and greater cross-talk between wells. Opaque white tape may be used to decrease luminescence loss and cross-talk.

ATP Contamination: Strict aseptic technique is essential to prevent ATP contamination of the CellTiter-Glo® 3D Reagent. Wear gloves and avoid contact with potentially contaminated surfaces and equipment. Clean gloves, lab surfaces and equipment with a 10% bleach solution, then pat dry with lab wipes (e.g., Kimwipes® tissues). Use individually wrapped or designated ATP-free pipettors and pipette tips whenever possible, and avoid inserting pipettors or pipette tips into the CellTiter-Glo® 3D Reagent bottle multiple times. Discard any unused, dispensed reagent; do not return it to the original bottle.

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5. Related Products

ATP Standard

Product	Size	Cat.#
rATP, 10mM	0.5ml	P1132

Cell Proliferation Assays

Product	Size	Cat.#
CellTiter-Glo® 2.0 Assay (Luminescent; single, stable reagent)	10ml	G9241
	100ml	G9242
CellTiter-Glo® One Solution Assay (Luminescent, single reagent)	100ml	G8461
	500ml	G8462
CellTiter-Glo® Luminescent Cell Viability Assay	10ml	G7570
(Luminescent)	10 × 10ml	G7571
	100ml	G7572
	10 × 100ml	G7573
CellTiter-Fluor™ Cell Viability Assay	10ml	G6080
(Fluorescent)	5 × 10ml	G6081
	2 × 50ml	G6082
CellTiter-Blue® Cell Viability Assay	20ml	G8080
(Resazurin; Fluorescent)	100ml	G8081
	10 × 100ml	G8082
CellTiter 96® AQ_{ueous} One Solution Cell Proliferation	200 assays	G3582
Assay (MTS; Colorimetric)	1,000 assays	G3580
	5,000 assays	G3581

Cytotoxicity Assays

Product	Size	Cat.#
CellTox™ Green Cytotoxicity Assay (Fluorescent)	10ml	G8741
	50ml	G8742
	100ml	G8743
CytoTox-Fluor™ Cytotoxicity Assay	10ml	G9260
(Fluorescent)	5 × 10ml	G9261
	2 × 50ml	G9262
CytoTox-Glo™ Cytotoxicity Assay	10ml	G9290
(Luminescent)	5 × 10ml	G9291
	2 × 50ml	G9292

5. Related Products (continued)

Cytotoxicity Assays (continued)

Product	Size	Cat.#
CytoTox-ONE™ Homogeneous Membrane	200–800 assays	G7890
Integrity Assay (LDH, Fluorometric)	1,000–4,000 assays	G7891
CytoTox-ONE™ Homogeneous Membrane Integrity Assay, HTP	1,000–4,000 assays	G7892
CytoTox-Glo™ Cytotoxicity Assay	10ml	G9290
(Luminescent)	5 × 10ml	G9291
	2 × 50ml	G9292
MultiTox-Fluor Multiplex Cytotoxicity Assay	10ml	G9200
(Fluorescent; Dual Assay)	5 × 10ml	G9201
	2 × 50ml	G9202
MultiTox-Glo Multiplex Cytotoxicity Assay	10ml	G9270
(Luminescent and Fluorescent; Dual Assay)	5 × 10ml	G9271
	2 × 50ml	G9272

Apoptosis Products

Product	Size	Cat.#
ApoTox-Glo™ Triplex Assay	10ml	G6320
	5 × 10ml	G6321
ApoLive-Glo™ Multiplex Assay	10ml	G6410
	5 × 10ml	G6411
Caspase-Glo® 2 Assay	10ml	G0940
Caspase-Glo® 3/7 Assay	2.5ml	G8090
	10ml	G8091
	100ml	G8092
Caspase-Glo® 6 Assay	10ml	G0970
	50ml	G0971
Caspase-Glo® 8 Assay	2.5ml	G8200
	10ml	G8201
	100ml	G8202
Caspase-Glo® 9 Assay	2.5ml	G8210
	10ml	G8211
	100ml	G8212
Apo-ONE® Homogeneous Caspase-3/7 Assay	1ml	G7792
	10ml	G7790
	100ml	G7791

Oxidative Stress and Metabolism Assays

Product	Size	Cat.#
GSH-Glo™ Glutathione Assay	10ml	V6911
	50ml	V6912
GSH/GSSG-Glo™ Assay	10ml	V6611
	50ml	V6612
Mitochondrial ToxGlo™ Assay	10ml	G8000
	100ml	G8001
ROS-Glo™ H_20_2 Assay	10ml	G8820
NAD/NADH-Glo™ Assay	10ml	G9071
	50ml	G9072
NADP/NADPH-Glo™ Assay	10ml	G9081
	50ml	G9082
NAD(P)H-Glo™ Detection System	10ml	G9061
	50ml	G9062

6. Summary of Changes

The 11/15 version of this document was revised to remove expired patent statements.

[a]U.S. Pat. Nos. 7,083,911, 7,452,663 and 7,732,128, European Pat. No. 1383914 and Japanese Pat. Nos. 4125600 and 4275715.

[b]U.S. Pat. Nos. 7,741,067, 8,361,739, 8,603,767, Japanese Pat. No. 4485470 and other patents pending.

[c]U.S. Pat. Nos. 6,602,677, 7,241,584, 8,030,017 and 8,822,170, European Pat. No. 1131441, Japanese Pat. Nos. 4537573 and 4520084 and other patents pending.

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